Role of Transvaginal Colour Doppler in Cases of Primary Infertility as Compared with Endometrial Biopsy

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(OBSTETRICS & GYNAECOLOGY)

M.L.B. MEDICAL COLLEGE
BUNDELKHAND UNIVERSITY,
JHANSI (U.P.)

2003 **NEERJA SACHDEV**

CERTIFICATE

This is to certify that the work entitled ***"Role of Transvaginal Colour Doppler in cases of Primary Infertility as compared with Endometrial biopsy"***, which is being submitted as a thesis for M.S., (Obstetrics & Gyanecology) examination, 2003, Bundelkhand University by Dr. Neerja Sachdev, has been carried out in the department of Obstetrics & Gyanecology, M.L.B. Medical College, Jhansi under my direct supervision and guidance. The observations recorded have been checked and verified by me from time to time.

Dated : 28.4.03.

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She has fulfilled the necessary stay in the department as required by the regulations of Bundelkhand University, Jhansi.

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Contents

Introduction

INTRODUCTION

Infertility is one field which has been revolutionised greatly both in diagnostic as well as therapeutic modalities in the last three decades. However, despite improved diagnostic modalities the factor leading to infertility in nearly 10-15% in rigorously evaluated couples remains unexplained. The treatment of unexplained infertility remains a challenge.

The main causes of infertility are anovulation in 10-30% cases, tubal factor in 15%, male factor in 20-30% cases, cervical factor in 25%, endometriosis in 5-25%, and unexplained causes in 15-30% of cases.

Poor quality embryo, higher age of the patient, unexplained infertility and unfavourable endometrium are some of the factors for implantation failure following IVF-ET reported in literature.

Good uterine perfusion combined with the rapid growth of the coiled arterioles and abundant angiogenesis in the luteal phase reflect the preparation of suitable environment for embryo implantation during each menstrual cycle. Blood flow in the follicular phase is presumed to be a fundamental pre-requisite for structural changes and increased vascularisation in the endometrium; and estradiol secretion in the follicular phase primes the endometrium. Furthermore, hormonal concentrations in follicular phase may also affect the uterine artery impedance directly and/or indirectly.

The physician's initial encounter with the infertile couple is the most important one because it sets the tone for subsequent evaluation

and treatment. Factors from either or both partners contribute to difficulties in conceiving; therefore, it is important to consider all possible diagnosis before persuing to invasive treatment.

Once the uterine lesion is suspected clinically, sonography can be used to establish the extent of lesion by studying the endometrial pattern and vascularity. The diagnosis can be further helped by endometrial biopsy.

Transvaginal sonography is a non invasive technique that has greatly improved ultrasonographic imaging over those taken via abdominal route. It's like "marrying palpation to imaging", and with the newer finger tip probes, this may even become the first step examination in obstetrics and gynaecology. It has changed the very practice of infertility and human reproduction, and today we cannot even think of practising infertility without TVS.

The primary advantage of TVS over conventional transabdominal sonography is the ability to place the higher frequency transducer nearer to the region of interest, permitting optimal visualization of the uterus, endometrial pattern, its thickness and echogenecity; cervix, ovaries, adenexal regions and cul-de-sac.

In combination with colour flow imaging and Doppler duplex, TVS provides a dramatic new insight into the physiological process and pathological conditions involving the female genital organs.

Goswamy et al (1988) reported poor uterine blood flow during the luteal phase in women with repeated failures of IVF-ET.

Steer et al (1992) measured uterine blood flow impedance just before embryo transfer and observed poor implantation rates in cases in which pulsatility index was higher i.e. > 3.0.

However, ***Schwartz et al*** (1997) did not find uterine artery Colour Doppler imaging parameters to be predictive of pregnancy outcome in IVF and ET cycles.

Wada et al (1994) demonstrated that the addition of low dose to standard hormone replacement therapy in women with impaired uterine perfusion undergoing frozen embryo replacement improved uterine blood flow, and thus enhanced implantation rates.

Inspite of unremarkable improvements in follicular stimulation, embryo culture, oocyte retrieval, fertilization and embryo transfer, there is discrepancy in the fertilization rates (90%) and implanation rates (10-15%) per embryo transfer in IVF cycles.

Thus, it has been realized that the receptivity of the uterus for achieving pregnancy either by natural means or assisted reproductive techniques is of profound importance and depends on several factors, many of which are yet to be identified.

Various studies have attributed uterine receptivity to the optimal changes involving endometrial thickness, endometrial echotexture, pattern, uterine perfusion, estrogen and progesterone receptor content of endometrium, levels of circulating estrogen and progesterone during various phases of menstrual cycle.

Aims and Objectives

AIMS AND OBJECTIVES

Present study is being carried out to study the efficacy of TVS Colour Doppler for studying the uterus, especially the endometrium and correlate them with histopathological findings. The study is contemplated with the following aims and objectives –

1. To study the uterine biophysical profile using various parameters of uterine scoring system for reproduction ***"USSR"*** by TVS in cases of primary infertility.
2. To evaluate the uterine blood flow using parameters like pulsatility Index ***(PI)*** and Resistance Index ***(RI)*** by colour doppler TVS.
3. To study the endometrial histopathology in primary infertility cases.
4. To make a comparative analysis of endometrial study by TVS with histopathological findings i.e. endometrial biopsy.

Review of Literature

REVIEW OF LITERATURE

Infertility is a problem which may encompass multiple etiologies involving either female or male partner, or both.

The etiology of infertility can be divided into three major categories –

(i) Female factor (40% cases), (2) male factor (40% cases), (3) remaining 20% are due to mixed male/female factors. In 10-20% of couples presenting for evaluation, no diagnosis can be made after standard investigation protocol (unexplained infertility).

The entire reproductive axis (hypothalamus, pituitary, ovary, pelvis, fallopian tubes, uterus and vagina) must be intact and integrated for the success of the reproductive system.

THE UTERINE FACTOR OF INFERTILITY :-

The uterine cavity must provide an environment for successful sperm migration from the cervix to the fallopian tubes. Normality of the mucosal lining, glandular secretion and vascularity are necessary to support implantation and placentation. Uterine anomalies, polyps, myomata, neoplasia, infections and intrauterine scar tissue can lead to poor reproductive performance.

Attempts have been made to correlate the sonographic parameters (such as thickness and reflectivity) and endometrial receptivity.

TVS has demonstrated to be a unique method of non-invasive assessment of normal and abnormal pelvic anatomy as well as early embryo development. It has allowed significant improvement in the

management of female infertility. It is a method of choice for monitoring follicular recruitment, maturation, rupture and corpus luteum formation in spontaneous and induced cycles as well as in in-vitro fertilization. In induced cycles, it also allows evaluation of possible complications. Moreover, in IVF it helps in safe and easy oocyte retrieval.

Recently, TVS in combination with pulsed colour doppler (Doppler triplex system – TVS + pulse Doppler + Colour Doppler) has been used in determining uterine and ovarian blood flow in spontaneous and induced cycles. This examination technique may find inadequate vascularization in even those patients of infertility who have normal uterine and ovarian morphology, thus explaining "the unexplained". Continuous and plused doppler waveform ultrasound can add dynamic information about the pelvic circulation, which cannot be obtained with imaging alone. Colour doppler has been used in in-vitro-fertilization to measure uterine blood flow in order to assess uterine receptivity, depending on which the decision for embryo transfer and their number can be taken. It can be hoped that TVS-colour doppler will move from research avenue into a routine and daily practice in infertility departments.

ADVANTAGES OF TVS :-

The transabdominal approach to doppler study of pelvic blood flow has become obsolete after the introduction of transvaginal approach. The major disadvantage of transabdominal approach is the need for a distended bladder for visualization of pelvic anatomy, and problems faced with obesity, bowel gas and retroverted uterus.

The full bladder displaces the pelvic organs backwards and the distance between the transabdominal probe and vessels under investigation. This examination is therefore limited to the use of low pulse repetition frequencies that results in decreased measurement accuracy and increased artefacts. Bladder compression may also cause alterations in blood flow in small arteries; although ***Steer et al*** (1995) reported otherwise. Furthermore patients can rarely tolerate an uncomfortably full bladder long enough to complete what may be a time consuming doppler study.

Vaginal sonography alleviates the need for full bladder and is obviously the preferred route for pelvic blood flow studies. The axial resolution or the ability to distinguish two points is a function of transducer frequency, and it improves with increase in frequency of the probe. However, with higher frequencies there is greater attenuation and loss of depth at which structures can be imaged. Since the vaginal probe is located close to the pelvic structures under investigation, one can afford to have higher transducer frequencies without fear of attenuation of signals. Hence, the vaginal probe with freedom to select the optimal pulse repetition frequency offers better resolution.

Transvaginal colour doppler has superior accuracy and reproducibility, and facilitates visualisation of small vessels i.e. spiral and radial arterioles. Compared to endometrial biopsy, it is a non-invasive tool for assessment of uterine receptivity.

<u>DOPPLER ULTRASOUND</u> :-

In the reproductive organs, neoangiogenesis physiologically occurs around the time of ovulation and trophoblastic invasion leading

to decreased impedance in the vessels supplying these organs. Hemodynamic changes are considered to be essential in the cyclic events of the ovary and have been linked to the process of ovulation and corpus luteal function.

The changes in uterine blood flow under the influence of hormones have been linked to successful implantation of early conceptus.

Taylor et al (1985) for the first time pointed out the possibility of investigating ovarian and uterine blood flow by means of pulse doppler system with a transabdominal scanner.

PRINCIPLE OF DOPPLER :-

Doppler ultrasound is based on the "Doppler Principle", named after Christian Andreas Doppler. It utilizes the changes in frequency of the incident and returned ultrasound beam that occur when sound waves are scattered from objects moving relative to the source of sound.

The changes in frequency of sound scattered from RBC's in circulation is utilized in estimation of blood flow velocities.

The frequency of sound is expressed in Hertz, named after Heinrich Rudolf Hertz. One Hertz is one sound wave (pulse) cycle occurring in 1 sec. Sound with a frequency of $\geq$ 20,000 Hz is ultrasound, because it is beyond the frequency range of human hearing. Colour flow doppler provides two dimensional colour coded information superimposed on the real time anatomic display. In doppler ultrasound, the change in frequency of returning echoes with respect to emitted frequency is called as "Doppler shift". This is proportional to the relative velocity of the target and to the cosine of the angle between

the source of wave and displacement axis of the target. The relationship is expressed by the doppler equation :-

$$F_d = 2\ F_s \operatorname{Cos} \theta\ \upsilon/c$$

where :

F_d is the Doppler shift frequency

F_s is the frequency of source

θ is the angle between the incident ultrasonic wave and the axis of the target (angle of insonation)

υ is the target velocity

c is the wave velocity in the medium.

Therefore, if the angle of insonation (θ) is maintained, Doppler shift (υ - d) will vary in direct proportion to the target velocity (υ).

Doppler shifts are usually in the range of 100 Hz to 11 KHz. The doppler shift is dependent on the speed of blood flow, the angle between the source of sound, direction of blood vessel being imaged and operating frequency of the doppler probe. Higher operating frequency, greater flow speeds and smaller doppler angles produce larger doppler shifts.

Ultrasound transducers operate on the principle of peizoelectricity by which certain materials produce a voltage when deformed by applied pressure, and produce a pressure when voltage is applied. Various formulations of lead zirconate titrate are commonly used in transducer source. Transducers operating in a continuous mode are driven by an alternating voltage and produce an alternating pressure that propagates as a sound wave. The frequency of sound called resonance frequency is equal to the frequency of the driving voltage.

For each pulse of ultrasound, a series of echoes are returned as the ultrasound pulse is reflected off objects at a greater or lesser distance. Doppler instruments are designed to recognize and display the differences in transmitted and received ultrasonic frequencies. These echoes are received by the transducer and converted into electrical energy, which is processed electronically and displayed as a series of dots in a single scanned line on the display.

CONTINUOUS WAVE DOPPLER (CWD) :-

It utilizes separate voltage generaters and receivers to create a picture of the Doppler shifts in the area being scanned similar to the gray scale. Continuous wave systems provide motion and flow information without depth information or selection capability. The frequency shift signals returned cannot be separated in order to examine a particular area. Therefore, it is mainly used in superficial vascular analysis and is obstetrics where the anatomical position of the vessel of interest is sufficiently isolated from moving neighbouring structures and there is little or no interference.

Pulse Doppler System has the ability to measure velocity selectively at specific locations in the path of the transmitted beam i.e. to the select the depth from which doppler information is received, allowing analysis of blood flow within a single vessel. To do this, the vessel to be studied is first located with colour flow doppler. Next, a gate is placed over the vessel which passes only signals that are returned within a defined time. The width of the gate (also called volume box) is adjusted to the diameter of the vessel. The returning doppler frequency shift echoes are converted electronically by a mathematical technique called Fast Fourier

transformation and displayed as Doppler shift Vs time waveform. The colour code of blood flow is red for towards the transducer, and blue away from transducer.

The doppler waveform represents changes in velocity of the blood flow during the cardiac cycle. Flow conditions downstream, particularly distal flow impedance is indicated by the relationship between peak systolic and end diastolic flow speeds.

In vessels like aorta and large vessels with valves, continuous forward flow occurrs due to elasticity of vessel wall – "Wind Kessel effect". In iliac arteries (with no valves), reversal of flow occurs during early diastole (triphasic flow). In ascending uterine artery, the early diastolic flow may be absent or reverse during menses or early proliferative phase, but this should not occur in the periovulatory phase (mid cycle). Late diastolic flow may be absent due to low pulse pressure or increased interval between two heart beats, and is less significant.

METHODS OF BLOOD FLOW ANALYSIS :-

Blood flow can be analysed in three ways –

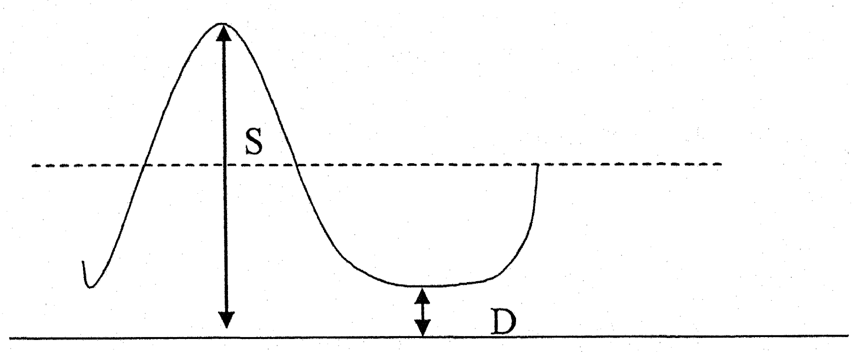
- ***Waveform***
- ***Resistance indices***
- ***Blood volume or velocity***

WAVEFORM ANALYSIS :-

Waveform analysis provides the most accurate estimate of blood flow when blood flow is absent in any time of the cardiac cycle. It utilizes descriptive terms, but because it is not subject to computer analysis, it is too often omitted. It is a gold standard for assessment of uterine and umbilical blood flow in second and third trimester.

Absence or reversal of early diastolic flow in umbilical artery is associated with IUGR in 84%. Persistence of early diastolic notch, especially in both uterine arteries is associated with maternal hypertension and IUGR.

RESISTANCE INDICES :-



They measure downstream impedance to blood flow, but are indirect estimate of blood flow and highly inaccurate when blood flow in the vessel is not continuous throughout the cycle. These are however preffered in small tortuous vessels because they are independent of angle of insonation. Another advantage is that they cancel out the error caused by doppler angle, machine gain and hypotension or hypertension, but are affected by heart rate.

RI (Pourcelot Index) – defined as the peak systolic minus end diastolic flow velocity divided by the peak systolic flow. It ranges from 1.0 to 0, with 1.0 forward flow. Therefore, it is suitable for low resistance beds with continuous flow throughout the cycle. Resistance index depends on the age, phase of the menstrual cycle, and specific conditions such as pregnancy and uterine tumors.

(PI) Pulsatility Index – is defined as peak systolic flow minus end diastolic flow velocity divided by average velocity. Although PI is not as accurate as RI because of variability inherent in software in measurement of average velocity, it can be used when blood flow is absent in all or part of the cardiac cycle.

SD Ratio – is simplest of all the indices and it is expressed as S/D ; where 'S' is the peak systolic flow velocity and 'D' is the end diastolic flow velocity. It is less frequently used, now that built in software programs can readily calculate PI and RI.

Error in the SD ratio increases as diastolic velocity becomes small. When flow is reduced in a stepwise fashion, the SD ratio increases exponentially, whereas PI and RI increase linearly.

FLOW VOLUME AND MEAN VELOCITY – They describe the actual volume of blood utilized by an organ. It is closest to actual blood flow, but difficult to perform because it is dependent upon the angle of insonation, accurate measurement of vessel diameter, tortuosity of vessel and the analytical power of the instrument. It can be used only for largest vessels. Uterine artery has a high to moderate flow velocity.

THE ENDOMETRIUM :-

Sonographically, the endometrium is one of the most dynamic structures in the body. During the reproductive years of a normal female, the uterus undergoes ultrasonographically detectable alterations characterized by cyclical changes in the echopattern of the endometrium. In fact, it is possible to infer the approximate day of a normal woman's menstrual cycle by the sonographic appearance of the endometrium.

From the first day of menstrual cycle until the midcycle, the normal endometrium progressively thickens and develops sonographically detectable strata. This appearance can be described as layered, trilaminar or 5-line; past the mid cycle, the normal endometrium brightens and progressively thins. These sonographic endometrial patterns appear to be related to the changes in the glandular and vascular elements of the endometrium during the menstrual cycle.

Fleischer et al (1986) determined that the endometrium is thickest during the secretory phase (3.6 $\pm$ 1.4mm); less thick during the proliferative phase (2.9 $\pm$ 1.0 mm), and thinnest during menstruation. These values are for the half thickness as measured from the endometrial canal to the endometrial-myometrial junction in A-P diameter. Full thickness measurement ranges from 4 – 12 mm, with an average thickness of 7.5 mm.

In prepration for implantation, the endometrium undergoes transformations influenced by the ovarian hormones produced during the early secretory phase – ***Michael Applebaum*** (1998). In the proliferative phase, estrogen affects uterine perfusion with a reduction in

vascular resistance of uterine arteries; incidentally this reduction seems to be larger in the artery ipsilateral to the dominant follicle. After ovulation, presumably in response to progesterone, a further decrease in vascular impedance is observed that is maximum in mid-luteal phase; during the peri-implantation phase. According to ***R. Fanchin et al*** (2001), the uterus seems to be a priviledged target for these cyclic vascular adaptations which also involve possible additional vasoactive processes including neuronal factors, prostaglandins and L-arginine - nitric oxide pathways.

These changes in endometrial pattern are due to an increase in the rate of blood flow, an increase in the number of cells populating the stroma and epithelium, an increase in uterine oxygen consumption, an increase in oxygen diffusion into the uterine lumen, generalized oedema, progressive increase of mucous secretions and coiling phenomenon of endometrial glands. These events take place from the endometrial base towards the surface.

Smith et al (1984) devised a grading system for the endometrium based on its reflectivity. This system compares the endometrium with the surrounding myometrium to determine the grade. The grading system further subdivides and characterizes the follicular phase endometrium as follows :-

Grade D – An almost anechoeic endometrium with a prominent mid line echo (triple-line) is characteristic of an early follicular phase endometrium.

Grade C – A 'darker' appearing solid area of decreased reflectivity (when compared with the 'lighter' myometrium) characterizes the mid-follicular phase endometrium.

Grade B – In late follicular phase endometrium, the endometrial reflectivity is similar to the myometrial reflectivity.

Grade A – A bright hyperechoeic endometrium characterizes the luteal / postovulatory period.

Gonen and Casper (1990) studied the endometrial texture and thickness during ovulation induction for IVF and described three different types of pattern. They found that triple-line endometrium was more likely to be associated with successful implantation, than the other two types (homogenously hyperechoeic or intermediate isoechogenic pattern). Furthermore, the endometrial thickness was greater in patients who became pregnant (8.7 $\pm$ 0.4mm) than in the group who did not (7.5 + 0.2 mm).

Kepic et al (1992) determined that endometrial thickness and pattern, follicle size and estradiol levels correlated to both the likehood of pregnancy and subsequent outcome.

Li and Colleagues (1987) examined the prevelance of abnormal endometrial development during the luteal phase of an infertile population (n = 142) and a fertile population (n = 68). The authors used histological dating by traditional criteria. The prevelance of a retarded endometrium was significantly higher in infertile than fertile couple (14% Vs 4.4%). The authors subdivided the infertile patients into 4 subgroups according to the cause of infertility. Patients suffering from endometriosis had a signficnatly higher prevelance (29%) of abnormal endometrial development; while no difference occured in patients with

tubal or male infertility. Furthermore, 21% of patients with unexplained infertility had out of phase endometrium.

Shulman et al (1987) used endometrial thickness to predict oestrogen status in women with amenorrhoea. Patients with an endometrial width of $\leq$4 mm reported scanty or no bleeding after administration of progesterone to induce menses; whereas patients with an endometrial thickness $\geq$ 5mm responded to progesterone with normal menstrual bleeding.

The endometrial thickness is measured through the central longitudinal axis of the uterine body on both sides of the midline. Increased thickness is associated with higher levels of estrogen, with an increased number of preovulatory follicles and with higher rates of fertilization and conception in IVF and ovulation induction cycles. An endometrial thickness of <5 mm is usually seen in the early to mid-follicular phase. At the time of ovulation, usually a thickness of 10–14mm is apparent. After ovulation, triple-line appearance, characteristic of follicular phase disappears and hyperechoeic endometrium wider that 13mm can be detected. The luteal phase endometrial appearance may be out of phase with timing of ovulation, indicating a luteal phase defect. This can be supported by hormonal assay or endometrial biopsy.

Deichert et al (1986) used the sonographic measurement of the midluteal endometrium as an indicator of luteal function in patients with infertility. If the endometrial thickness was less than expected, they recommended a hormonal evaluation.

AGE AND ENDOMETRIAL FACTOR :-

Navot and Edwards et al (1991) addressed the relationship between increasing maternal age and a decline in fertility. The main determinant of reproductive outcome in this age group is oocyte quality rather than endometrial receptivity. ***Batista et al*** (1995) proved normal luteal and endometrial secretory function and normal endometrial maturation in cycling women aged over 40 years. Their results clearly indicated that implantation failure due to a hostile endometrium does not play a significant role in the decline of fertility in this population.

Kurjak and Kupesic et al (1995) performed serial measurements throughout the menstrual cycle in 120 normally cycling women with documented infertility, 85 post menopausal patients and 45 post menopausal patients receiving hormone replacement therapy. They concluded that ageing process initially affects uterus less than the ovary. Furthermore, the uterine environment could be easily manipulated during the menopausal years by proper hormonal stimulation.

ENDOMETRIAL PERISTALSIS :-

Birnholtz et al (1984) was the first to report endometrial movements as a reflection of myometrial activity. These contractions are most common in follicular phase and culminate around ovulation. Subendometrial contractions propagate from the internal cervical os to the uterine fundus during the periovulatory period, but reverses during menses. Such directional contractions assist sperm transport and the asymmetric waves serve to maintain the blastocyst within the uterine fundus.

Abramowitz and Archer and De Vries (1990) made a classification of movements in terms of intensity and frequency using a transvaginal probe. The ideal method to observe this peristaltic motion is by recording the examination on videotape and later playing back the tape at accelerated speed.

Oike et al (1992) correlated endometrial activity with hormonal levels. They found that endometrial peristalsis has a strong correlation with estradiol levels. Rising progesterone levels seem to reduce the frequency of endometrial/myometrial movements.

UTERINE BLOOD FLOW IN NORMAL CYCLE :-

The follicle and corpus luteum of ovary and endometrium are the only areas in normal adult women where angiogenesis occurs. Blood flow in pelvic blood vessels, including the spiral and radial vessels has been studied in relation to different stages of menstrual cycle. The main uterine artery and vein are best depicted at cervico-corporeal junction. The vessels are lateral to the uterine corpus. Various authors have studied the uterine artery blood flow during normal menstrual cycle.

Goswamy et al (1988), the pioneer in this field; and ***Steptoe*** (1988) were the first to suggest that abnormal uterine artery blood flow might be associated with infertility.

They compared the RI and waveform characteristic of multiparous patients with nulliparous patients in natural cycles and found that multiparas had continuous diastolic flow throughout most of their cycle, except a few days postovulation and during menstruation. In nulliparas, RI fell with rise in Estrogen during early follicular phase, then rose when Estrogen fell in the immediate postovulation period. A

second decrease in RI occurred in mid luteal phase with rising serum Estrogen and Progesterone concentration. In nulliparas, the values were markedly different on days 14 and 21, which may have been due to the fact that waveforms were not continuous in them on most days, or due to real differences in vascular development and blood flow following completion of a full term pregnancy. It was concluded that normal uterine blood flow values must be obtained from patients with known fertility and ability to carry to term. Also, normal values can only be obtained from studies in which flow is continuous throughout the cardiac cycle.

Taylor et al (1985) in their classic study measured the uterine artery blood flow using abdominal ultrasound as well as by an invasive technique employing 10 MHz directional continuous wave flow meter applied directly to the ovarian, iliac and uterine artery. They found that uterine artery waveforms and PI were identical for both techniques and that PI was lower on the side with ovary bearing the dominant follicle. They also studied the changes in PI longitudinally along the menstrual cycle and found an increase in blood flow in the uterine artery on the dominant side as early as day 5 of the cycle.

Scholtes et al (1989) found no significant variations in PI during normal menstrual cycle using transvaginal ultrasound, although it was highest on day 16. They reported forward flow velocity waveforms in the uterine artery in all patients. The PI of uterine artery was significantly lower on dominant than non-dominant side on day 21. These findings may be explained by increased uterine contractility and

compression of vessels traversing the uterine wall; which decrease their diameter and carries a consequent higher resistance to flow.

Steer et al (1990) found marked variation in uterine artery PI during the menstrual cycle, using TVS. Values were lowest 6 days before the LH peak and 9 days after the LH peak. The highest values occurred at the mid cycle and 3 days after LH peak. They found no difference in PI on the side of the dominant follicle like ***Kurjak et al*** (1993), who measured uterine artery PI during periovulatory period, using TVS. In 12% patients in ***Kurjak's*** study (1993), diastolic flow was absent in both uterine arteries. The PI was 3.14 two days before ovulation and nadir of 2.22 was reached, a day before ovulation. In their study in 1991, RI was measured throughout the length of menstrual cycle and fall of RI from 0.88 ± 0.04 (in proliferative phase) to 0.84 ± 0.04 on day 18, was noted. Forward diastolic flow was seen in almost all patients.

Sladevicius (1993) measured uterine artery PI using TVS. Throughout the cycle, continuous forward flow was present in all the patients. PI was highest 2 days after LH surge and lowest on days 7 and 12 postovulation. There was a difference between PI of dominant and non-dominant side in the periovulatory and luteal phase. ***Salle B*** (1994) assessed blood flow in uterine artery by TVS, using RI as the reference. The RI of uterine artery was 0.43 during the follicular phase, 0.50 during the ovulatory peak and 0.41 during the luteal phase. These variations were significant.

de Zeigler (1991) studied the uterine artery PI in young women with inactive ovaries who were given transdermal estrogen and

progesterone (vaginal) after a 28 day replacement regimen duplicating estrogen and progesterone levels seen in natural menstrual cycle. In mid-cycle, at the time of estrogen peak, doppler waveforms showed a marked broadening with uninterrupted diastolic flow indicative of a profound decrease in PI to 1.3 $\pm$ 0.3. Doppler measurement made on days 26 and 27 i.e. immediately premenstrual period when hormonal levels were at the lowest showed no significant change (1.7 $\pm$ 0.3). This appears to indicate that the decrease in vascular resistance observed at the time of ovulation in menstrual cycle is meditated by estrogen, and that progesterone addition in the luteal phase does not interfere markedly with the vasodilative effect of estrogen on uterine arteries.

Tan et al (1996) in their study of 7 patients with unproven fertility found that the uterine PI was significantly lower (3.05 Vs 2.34) and TAMV higher (16.3 Vs 13.2 cm/s) on the side of the dominant follicle during mid-luteal phase, but no significant changes in either measurement during the follicular phase of the cycle.

Brannstromm et al (1998) in their cross-sectional study of 24 women awaiting IVF in natural cycle in terms of PI and RI in uterine arteries on 10-12 days of menstrual cycle, found that the indices of blood flow were similar in the left and right uterine arteries with no obvious relation to the laterality of the dominant follicle and found no difference before or after LH surge.

Zaidi et al (1995) assessed uterine artery blood flow in 6 women during periovulatory period. They found that mean uterine artery PI showed a marked daily fluctuation with the nadir occurring most commonly at 6.00 a.m. after overnight rest in both dominant and non-

dominant uterine arteries. They found no temporal relationship between the fluctuation in PI and changes in LH, FSH, Estradiol, Progesterone concentration. These findings suggested that there is a circadian rthym in uterine artery blood flow during periovulatory period which appears to be independent from the hormonal changes.

ENDOMETRIAL BLOOD FLOW :-

Changes in endometrial vascularity appears on colour doppler examination which may reflect the histologic changes described by the pathologists. If one divides the endometrial and periendometrial areas in the following four zones :-

Zone – 1 : A 2 mm thick area surrounding the hyperechoeic outer layer of endometrium.

Zone – 2 : The hyperechoeic outer layer of endometrium.

Zone – 3 : The hypoechoeic inner layer of endometrium.

Zone – 4 : The endometrial cavity.

It is possible to see variations in the depth of vascular penetration before, during and after the mid-cycle. Most patients without diagnosed infertility (presumed normal) usually demonstrate flow into zone 3 by the mid-cycle –***Michael Applebaum*** (1998-1999).

Vascular penetration towards the endometrial canal differs among patients. In patients with uterine artery PI's of less than 3.0, there are no reports of successful pregnancies in IVF patients, unless there is vascularity demonstrable within zone 3 or within zone 3 and 4 prior to

transfer. Colour doppler findings in unsuccessful cycles may relate to the histologic findings described by ***Sterzik et al*** (1989). In their study of 58 IVF patients, a majority demonstrated an immature endometrium at the time of embryo transfer. The abnormalties included a variety of patterns, all indicating a lack of secretory transformation, suggesting unpreparedness for implantation.

Wiczyk et al (1998) have speculated that the increased estradiol levels during the proliferative phase have an angiogenic effect on the junctional zone vasculature. The increased vascularity provides the nutrient and hormonal stimulation necessary for endometrial development.

Yang et al (1999) undertaking a qualitative assessment of intraendometrial vascularization identified a critical endometrial surface ($5mm^2$) under which endometrial receptivity might be hampered.

POSTURAL EFFECTS ON UTERINE BLOOD FLOW :-

A consistent limitation of Doppler ultrasound studies of blood flow is that, measurements have been obtained only in recumbent position. Blood flow measurements while standing may be equally or more important physiologically, and may differ significantly from recumbent measurements. Standing causes a change in position and/or downward movement of the uterus, which may result in acute angulation or stretching of uterine artery especially in patients with poor uterine support. As a consequence arterial diameter may decrease, and if uncompensated by increased pulse rate or decreased downstream resistance can result in decrease in blood flow volume.

Dickey et al (1994) showed that standing 9-14min. reduced the uterine artery flow volume by 34% and RI increased in 70% of the population.

<u>THE UTERINE BIOPHYSICAL PROFILE</u> :-

Certain sonographic qualities of the uterus are noted during the mid-cycle. These include –

1. Endometrial thickness in greatest A-P dimension of 7 mm or greater (full-thickness measurement).
2. A layered ("5 line") appearance to the endometrium.
3. Blood flow within zone 3 using colour doppler technique.
4. Myometrial contractions causing a wave like motion of the endometrium.
5. Uterine artery blood flow, as measured by PI, less than 3.0.
6. Homogenous myometrial echogenecity.
7. Myometrial blood flow seen on gray-scale examination (internal to the arcuate vessels).

The uterine scoring system for reproduction ***("USSR")*** comprises evaluation of the following parameters.

1. Endometrial thickness (full thickness measured from the myometrial–endometrial junction to the endometrial–myometrial junction).
2. Endometrial layering (i.e. a 5-line appearance).
3. Myometrial contractions (seen as endometrial motion).
4. Myometrial echogenecity.
5. Uterine artery doppler flow evaluation.

6. Endometrial blood flow.
7. Gray-scale myometrial blood flow.

Each parameter is scored as follows –

1. Endometrial thickness
   a. < 7mm = 0
   b. 7 – 9 mm = 2
   c. 10 – 14 mm = 3
   d. > 14mm = 1

2. Endometrial layering
   a. no layering = 0
   b. hazy 5-line appearance = 1
   c. distinct 5-line appearance = 3

3. Myometrial contractions (seen as wave – like endometrial motion high speed play back from videotape).
   a. < 3 contractions in 2 min (real time) = 0
   b. > 3 contractions in 2 min (real time) = 3

4. Myometrial echogenecity
   a. coarse/inhomogenous echogenecity = 1
   b. relatively homogenous echogenecity = 2

5. Uterine artery doppler flow evaluation
   a. PI > 3.0 = 0
   b. PI < 2.5 – 2.99 = 0
   c. PI < 2.2 – 2.49 = 1
   d. PI < 2.19 = 2

6. Endometrial blood flow within zone 3

   a. absent = 0

   b. present, but sparse = 2

   c. present, multifocally = 5

7. Myometrial blood flow internal to the arcuate vessels seen on gray scale examination

   a. absent = 0

   b. present = 2

The values assume a technically adequate ultrasound examination with no abnormalities of uterine shape or development, no other gross uterine abnormalities (e.g. significant masses), and a normal ovarian cycle (e.g. without evidence of ovarian uterine discordination). A male factor component to the infertility is not present.

USSR "perfect score" of 20 has been associated with conception 100% of the time. Scores 17 – 19 have been associated with conception rates 80% of the time. Scores 14 – 16 have a 60% chance, while scores 13 or less have resulted in no pregnancies.

Absent endometrial flow; despite highest values for the other parameters has always been associated with no conception.

UTERINE RECEPTIVITY :-

The importance of uterine receptivity as a crucial factor in infertility was realized when inspite of improvements in quality and techniques of embryo transfer, with fertilization rate as high as 90%, the implantation rate per embryo remained as low as 10-15%. Maximum of upto 30% has been achieved with multiple embryo transfer. Thus,

implantation is a major rate limiting step in IVF. Uterine receptivity is of great importance in successful implantation. The correlation of uterine receptivity to successful implantation is as high as 31 – 64%, compared to embryo quality i.e. 21-32%. The paradox however is that although embryo quality can be assessed by standard embryological techniques, uterine receptivity has been assessed by biopsies in the past, but at the same time endangering the implantation site. It will give information regarding hormonal status; luteal defects, if any and ultrastructure examination will reveal the integrity of endometrial barrier. Lately, it has been realised that both endometrial development and uterine perfusion contribute to receptivity.

These changes in endometrial morphology and blood flow during the menstrual cycle can now be easily assessed by non-invasive modality of Doppler ultrasound.

There is much debate regarding the relationship of endometrial thickness and morphology to successful implantation. Some studies found no correlation between endometrial thickness and occurance of pregnancy. Many authors, on the other hand have reported significant correlation between pregnancy and endometrial thickness.

Most investigators have said that the positive predictive value of endometrial thickness is low. However, a threshold value of endometrial thickness has a grave negative predictive value for the subsequent occurrance of pregnancy, has been described by different workers.

Coulman 1994 (< 6 mm); ***Check*** 1993 (< 10 mm); ***Shapiro*** 1993 (<7mm); ***Smith*** 1984 (< 5 mm); ***Rabinowitz*** 1986 (< 13 mm); ***Olieviera*** 1997 and ***B Salle*** 1998 (< 7 mm). Echogenecity patterns of the

endometrium have been studied by various investigators, most of whom agree that a triple or mixed (incomplete) pattern can sustain pregnancy, while a homogenous pattern cannot; except a few.

Measurement of impedance to uterine artery blood flow in IVF cycle has provided indirect estimation of endometrial receptivity.

Goswamy et al (1988) found a poor blood flow, no or only transiently measurable diastolic flow, in about 48% patients with > 3 unsuccessful IVF attempts by transabdominal ultrasound, and also that the uterine perfusion, assessed by RI can be improved with administration of estradiol.

Sterzik (1989) found that RI was significantly lower at the time of oocyte retrieval, using transabdominal sonography, in subsequently pregnant patients undergoing ovarian stimulation.

Strohmer (1991) studied the PI, using TVS on the day of hCG administration and concluded that hCG administration can be withheld if the flow is not favourable. ***Steer et al*** (1992) determined a threshold PI value for the uterine artery using TVS colour doppler on the day of embryo transfer. They suggested a mean PI $\geq$ 3.0 on the day of embryo transfer could predict upto 35% patients who failed to become pregnant with a sensitivity of 100%. They, in fact suggested that an optimal uterine receptivity corresponds to PI of 2–2.99. If PI > 2.99, it indicates a hostile uterine environment. These females can be advised that pregnancy is unlikely in their treatment cycle. Therefore, cryo preservation can be done for transfer in subsequent favourable cycles which theoretically improves the pregnancy rates upto 50% per embryo transfer. For patients with optimal PI values, maximum 2 embryos

should be transferred to prevent multiple gestation which can lead to dimnished perinatal outcome compared to singleton pregnancy. Furthermore, no studies have obtained implantation when PI $\geq$ 3.0. Also different threshold values have been suggested. ***Cacciatore*** (PI > 3.3); ***Serafini*** 1994; ***Sterzik*** 1989; ***Zaidi*** 1995; ***Bied Damon*** 1995 (PI > 3.5); ***Coulman*** 1995 (PI >3.3); ***Levi-setti*** 1995; ***Diechart*** 1996. If a threshold PI value for uterine artery is defined has 3, Doppler flow has high negative predictive value (100%) and sensitivity 100%. ***Supero et al*** (1993) drew similar conclusion as ***Steer et al*** (1992) using transabdominal ultrasound doppler assessment of uterine flow in selecting cycles for successful embryo transfer.

Basil (1995) measured uterine artery RI, using TVS from the day of HMG administration to embryo transfer. They concluded that RI > 0.79 before starting HMG was associated with a poor uterine response and suggested that increased dose of HMG might increase uterine blood flow and uterine receptivity. ***Serafini et al*** (1996) also found that patients who did not become pregnant had a significantly higher resistance to blood flow than those who did establish a successful pregnancy in stimulated cycles of patients undergoing ovulation induction for ART.

Triple-line endometrial pattern and diastolic flow were the only predictive markers of term pregnancy. ***Levi Setti*** (1995) measured mean PI from 5 days before hCG administration to day of oocyte retrieval in IVF patients. They found PI > 3.0 on day of hCG administration was associated with 24% pregnancy rates compared to 42% when PI was $\leq$ 3.0. ***Tekay et al*** (1996) measured PI and waveform type in IVF

patients using TVS. They found no difference in mean PI in conception and non-conception cycles among patients with continuous flow; although in conception cycles, individual PI was always <4.0. In patients with absent end diastolic flow, there were no conceptions.

Cacciatore et al (1996) measured uterine artery RI and PI, using TVS, on the day of embryo transfer. The mean RI and PI were significantly lower in pregnant than in non-pregnant patients. The pregnancy rates were 40%, 15% and 10% when mean PI values were < 3.0; ≥ 3.0 or ≥ 3.5, respectively. When RI was ≥ 0.92, pregnancy rates dropped at 13% from 39.5% when RI was ≤ 0.91. They concluded that the best cut off values were 3.3 for PI and 0.95 for RI. Absent diastolic velocity was noted only in 6 patients, one of whom conceived, but aborted.

In contrast to all the above studies, ***Bustillo et al*** (1995) observed no differences in endometrial thickness, PI or RI between conception and non-conception cycles. Hoverer, no pregnancy occurred when PI was ≥ 3.4. These findings were consistent with those of ***Tekay et al*** (1996) and ***Tekay et al*** (1995). The latter study also found a significant increase in uterine receptivity when PI was between 2 and 2.99.

Thus, a consensus of published work indicates that implantation in IVF cycles is decreased when uterine artery PI ≥ 3.3 and RI ≥ 0.95 or when waveforms show absence of early or end diastolic flow at the time of hCG administration, oocyte retrieval or embryo transfer. Thus, studies linking uterine blood flow to IVF outcome performed thus far strongly suggest that uterine blood flow parameters can help define which patients will become pregnant. A logical approach would be to

analyse all the ultrasound and Doppler parameters together in order to ascess uterine receptivity. ***Applebaum*** (1995)suggested a combination of different echographic parameters ***("USSR")*** to give a score defining uterine profile.

Following the pioneering work of ***Goswamy et al*** (1988) relating uterine blood flow and IVF, ***Kurjak et al*** (1991) and (1993); ***Fujimo et al*** (1993); ***Steer et al*** (1994) and ***Kuo et al*** (1997) investigated uterine blood flow in women with infertility during natural cycles.

Kurjak et al (1991) assessed uterine perfusion in 100 infertile women and 150 fertile women using TVS. They measured changes in uterine artery RI throughout the menstrual cycle and made a note of the waveform pattern. They found no difference in mean (Right and Left) uterine artery RI between infertile and fertile women. However they found that uterine artery blood flow decreased a day before ovulation. A fall of RI occurred from 0.88 ± 0.04 in proliferative phase to 0.84 ± 0.04 by day 18, and remained at this level for the rest of the cycle. No fall was noted in anovulatory cycles. On waveform analysis, end diastolic flow was absent in 12 women, of which 11 were infertile. They concluded that flow velocity changes begin before ovulation, and thus; are not purely secondary to progesterone action.

In 1993, they conducted another study, in which TVS colour Doppler was done to assess uterine flow in uterine and other vessels in a group of 78 infertile women in periovulatory period during spontaneous and induced cycles (from PI =3.16 to PI 2.22), which was absent in the stimulated cycle (3.06). There was no difference in blood flow regarding position of follicle. Absent diastolic flow was noted in 12 cases, 4

spontaneous and 8 induced. Endometrium was thickened in spontaneous cycle in comparison to clomiphene citrate cycle (2^{nd} or 3^{rd}), but thinner in comparison to HMG cycle. The final conclusion was that, better uterine perfusion occurs in the periovulatory phase in spontaneous as compared to induced cycles. Endometrial perfusion presents accurate assay of uterine receptivity to predict implantation success rate to reveal unexplained infertility and to select patients for correction of perfusion abnormalties.

A study by ***Fujimo et al*** (1993) on 60 infertile women commenced measurements of PI of uterine artery in follicular, ovulatory and mid-luteal phase of 13 stimulated cycles. A comparison of patients who conceived in treatment cycle to those who did not conceive was also done. There was a significant difference of PI in pregnant (1.67 $\pm$ 0.22) vs non-pregnant (2.30 $\pm$ 0.78) patients in follicular phase. The difference was also significant in pregnant cycles during follicular (1.67 $\pm$ 2.2) vs mid-luteal phase (2.33 $\pm$ 0.69), but not in ovulatory and mid-luteal phase. They hypothesized that a decrease in the impedance to blood flow in pregnant cycles was the evidence of importance of blood supply in follicular phase. The endometrial thickness in pregnant cycles was more than is non- pregnant cycles in mid-luteal phase. Thus, the blood flow in the follicular phase was presumed to be a fundamental prerequisite for structural changes and increased vascularization in the endometrium prior to successful conception.

Steer et al' 94 measured mean PI of uterine artery in mid-luteal phase is females with different causes of infertility during natural cycles, using transvaginal doppler, and a comparison was done with presumably

fertile women with azoospermic husband. The authors had earlier '90 established that impedance is lowest in mid-luteal phase in normal fertile non-pregnant population. In this study they made an attempt to establish whether the impedance was different in females with different causes of infertility. There was no significant difference in PI of right and left side as a mean value was taken, except in 4 patients in whom unilateral salpingectomy was done. In the latter, PI was higher on side of salpingectomy. The PI of infertile group was significantly higher than that of normal fertile group viz,1.9 in normal ; 2.45 in unexplained; 2.65 in tubal damage; and 2.32 in endometriosis; 3.03 in anovulation. A possible explanation for these findings was suggested. In patients of endometriosis or tubal damage, associated scarring and inflammation may inhibit the perfusion. In anovulatory patients, the cause may be the sub-hormonal levels on day 21. In patients with unexplained infertility, despite normal ovarian function, a normal pelvis on laparoscopy, the PI was outside the normal range seen in fertile women i.e. 0.84-2.95; thus supporting the notion that decreased perfusion in mid-luteal phase, the physiological time of implantation might be a cause of unexplained infertility. The mechanism mediating increased impedance may be an attenuation in the uterine artery response to the circulating ovarian hormones or an immunological factor.

Tinkanen et al (1994) studied uterine artery blood flow by using TVS doppler in infertile patients during spontaneous, ovulatory, menstrual cycles and compared with 19 fertile women with regular cycles. They measured pulsatility index in uterine arteries during follicular and mid-luteal phase of cycle. A higher PI in uterine arteries

was noted in infertile patients during the luteal phase as compared to fertile women.

SAFETY OF TRANSVAGINAL DOPPER ULTRASOUND :-

The effects of Doppler ultrasound on embryonic and foetal development are not known with certainity. Bioeffects of Doppler ultrasound are related to the spatial peak time, average intensity (**I_{SPTA}**) and duration of exposure.

The American Institute of ultrasound in medicine (1993) has recommended that Doppler ultrasound during early pregnancy can be limited to 500s at an **I_{SPTA}** $<94 mW/cm^2$.

ROLE OF DOPPLER IN INFERTILE POPULATION :-

Infertility is defined as one year of unprotected coitus without conception. It is estimated that 10-20% couples attending infertility clinics have unexplained infertility. Occult problems in oocytes and/or the spermatozoon can lead to fertilization failure/dysfunctional embryos. Infections, minimal tubal damage, leutinised unruptured follicle, peritoneal factors, endometrial receptivity and immune system are other proposed causes. Among these, the last two have been dealt with in great detail by various workers.

TVS in the work up of infertility is like a stethescope to a physician. Not only it tells us about the tubal patency, but ovarian folliculogenesis, uterine malformations, cervical factor and to some extent pelvic factors can be studied; this has today reduced the need of invasive endoscopy for infertility evaluation. Now, with TVS we can do a complete evaluation of infertility in a single menstrual cycle ***"Single cycle evaluation" (Malhotra and Malhotra)***. Here we stimulate a women from day 2 of menstrual cycle after a baseline TVS; do a

sonosalpingography on day 7; TVS follicular monitoring day 10 onwards; time the hCG injection when dominant follicle is 20mm; do a PCT after 24 hours of hCG and look for corpus luteum by TVS on day 16^{th}. During our follicular monitoring scans, we do the uterine scoring system for reproduction, as advocated by Chicago University ***"USSR"***. This will go a long way in predicting successful implantation in IVF, and to determine the date and time for embryo transfer to get the optimum success.

Laboratory methods, such as endocrine assays for reproductively active hormones or histologic examination of uterine biopsy specimens are routinely used; but are inconvenient, expensive and results are not immediately available. Two–dimensional ultrasonographic imaging and transvaginal doppler (particularly with addition of colour doppler imaging) permits rapid non-invasive visualization and immediate conclusions.

ADVANTAGES :-

- It is the first base line scan for detailed pelvic study and ovarian morphology.
- Daily follicle monitoring in stimulation protocols.
- Study of endometrial lining.
- Ovum pickup for ART's.
- Evaluation of tubes by sonosalpingography.
- Doppler and colour flow imaging.

LIMITATIONS :-

- Lesions outside short range of TVS may be missed.
- Maneouverability of probe in vagina in limited.
- Pre-pubertal and virgin patients.

- Vaginal stricture.
- Psychologic or personal reasons for not undergoing TVS (Medical rape).
- Poor acceptance in Indian orthodox patients (though recent study from Bombay and Agra show 100% acceptance in all social sections of Indian women).
- Theoretical risk of causing bleeding in pregnant patient.
- Cervical lesions may distort the picture.

Material and Methods

MATERIAL AND METHODS

The present study was carried out in the department of Obstetrics and Gynaecology in association with postgraduate department of pathology, MLB, Medical College, Jhansi. A total 100 subjects for study were chosen from :

(1) Patients coming for the first time to out patient Department of Obstetrics and Gynaecology with complaints of primary infertility.

(2) Primary infertility patients coming to outpatient department under regular follow up.

Criteria for selection of cases -

(1) Cases during reproductive period of life (18 to 40 years) were included in the study.

(2) Case with stenosed vagina were excluded.

All cases included in the study were postally interrogated, were subjected to detailed clinical history and examination.

All the patients underwent Transvåginal colour doppler sonography and endometrial biopsy in the same sitting.

HISTORY :-

Clinical History :-

Detailed clinical history was taken regarding :-

(a) Age.

(b) Duration of Marriage.

(c) Education Status.

(d) Income group.

(e) Residential area – Urban / Rural.

(f) History of previous marriage with proven fertility.

(g) Medical history, especially of tuberculosis, diabetes, sexually transmitted diseases.

(h) Surgical history especially of abdominal or pelvic surgery.

(i) Menstrual history in detail.

(j) Contraceptive practice especially of IUCD insertions.

(k) Sexual history with problems such as dyspareunia, loss of libido.

Treatment History :-

Patients were asked about the treatment given in the form of hormonal treatment to control menstrual irregularity, or a treatment taken for any sexually transmitted disease.

EXAMINATION :-

General Examination :

This included the general built of the patient, height, weight, pulse, blood pressure, respiratory rate, temperature, icterus, cyanosis, pallor, oedema, lymphadenopathy.

Systemic Examination :

A thorough systemic examination was done to exclude any systemic disease because some of them may be directly responsible for changes in menstrual pattern.

Per abdominal Examination :

Abdomen was examined for any lump or tenderness.

Local Examination :

Perspeculum Examination :- This was done to exclude any local pathology in vagina and cervix.

Bimanual Examination :

This was done to find out the position, size, shape consistency, mobility, tenderness of uterus and its appendages.

Reproductive system Examination :

- Distribution of hair.
- Development of secondary sexual characters.
- Examination to exclude chromosomal disorders like Turner's syndrome.

INVESTIGATIONS :

- Routine Investigations
- Blood

  Hb

  TLC

  DLC

  ESR
- Blood urea
- Complete urine examination
- VDRL — Husband / wife
- Blood sugar — fasting / post prandial
- TORCH
- Husband's seminogram.

ULTRASONOGRAPHY :

Patients were subjected to transvaginal sonography using a transvaginal probe to visualize the pelvic structures in a systemic manner.

Transvaginal probe :

The convex linear array Intravaginal transducer (CLAIVT 3.5) is a 3.5 MHz convex probe with a radius of curvature of 11.0mm.

Technique :

- Patient was put in lithotomy position after emptying the bladder.
- A brief description of transvaginal scanning and reason for performing it was offered to the patient.
- Scanning was performed with patient is supine position with her thighs abducted and knees flexed.
- The transvaginal probe was covered with a condom containing small amount of coupling gel.
- Additional gel was applied on outside of the sheathed tip.
- Disposable gloves were worn by the sonographer during probe insertion and scanning of patient.
- The gain and magnification were adjusted.
- The probe was gently pushed into vagina and basic manoeuvers to see the pelvis by
    - a) Tilting or angling the probe.
    - b) Pushing or pulling the probe.
    - c) Rotating the handle.

The structures in the pelvis were visualized in a systemic manner to minimize diagnostic errors.

The following sequence was followed –

(i) Cervix, cervical canal.

(ii) Uterus, body, fundus, endometrial canal and uterine arteries.

(iii) Pouch of Douglas for free fluid.

(iv) Lateral pelvic wall and iliac vessels.

(v) Adenexa.

(vi) Fallopian tubes.

(vii) Ovaries and ovarian follicles.

(viii) Other masses or abnormalties.

After scanning, the transvaginal probe was removed from vagina and the coupling gel was wiped off with damp towel or tissue paper.

TVS was done to study –

A) All the 3 dimensions of uterus.

a) Antero-posterior diameter.

b) Length of uterus.

c) Width (transverse scan) of uterus.

B) Myometrial echoes :- From the myometrium which included

a) Intensity of echoes.

b) Texture of echoes.

c) Homogenecity of echoes.

C) Endometrium :- Echoes from endometrium included –

a) Length of endometrial cavity, any collection or growth in cavity.

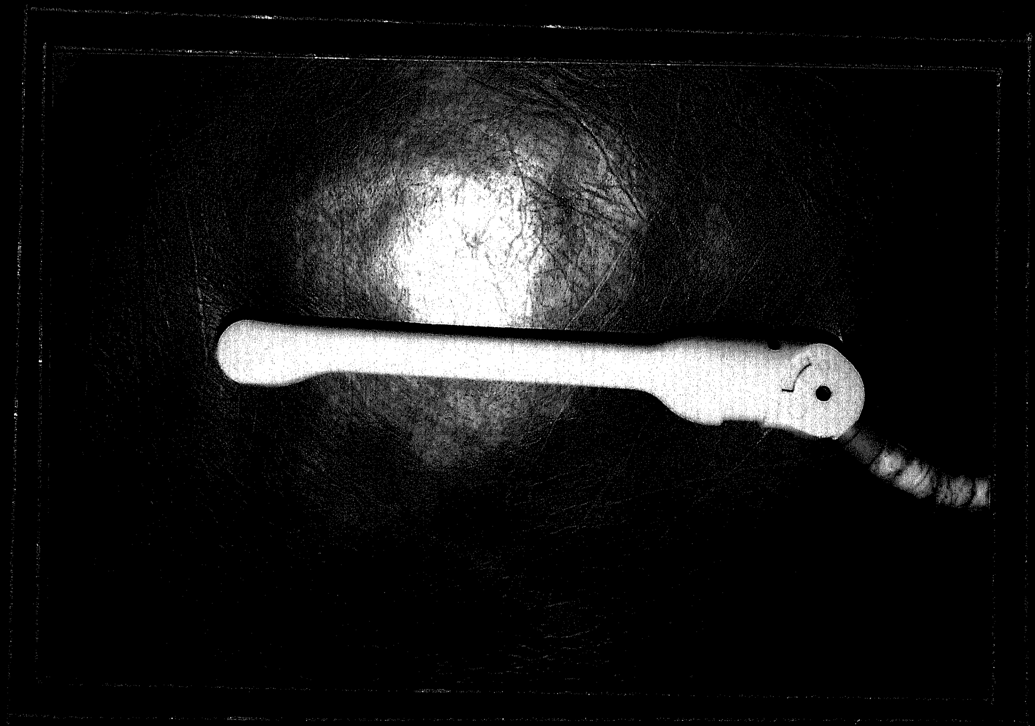
b) Thickness of endometrium.

c) Type and echocharacteristics of endometrium.

d) Endometrial-myometrial junction.

D) Ovaries for its size, shape, echotexture and number of follicles were studied.

E) Pouch of Douglas for any collection or growth.



TRANS VAGINAL PROBE

Colour Doppler Study - The vascular pattern of uterine artery during systole and diastole were studied to predict fertility outcome in infertile patients using following indices :-

1. SD Ratio = Peak systolic doppler shift frequency/Least diastolic doppler shift frequency
2. Pulsatility Index (PI) = S – D/Mean
3. Resistance Index (RI) = S – D/S

S = Peak systolic frequency shift, D = End-diastolic frequency shift
mean = mean height over one cardiac cycle.

ENDOMETRIAL BIOPSY – Endometrial biopsy was taken following ultrasound examination is all cases in the premenstrual phase and endometrium was studied for its histopathological findings.

Method :- Patient was put is lithotomy position after giving paracervical block. Vulva was painted by sponge holding forceps. Size of the uterus was assessed by bimanual examination. Sims speculum was then inserted and the cervix was visualised with the help of anterior vaginal wall retractor. The anterior lip of cervix was held with volsellum. Uterine sound was passed to assess the length of uterine cavity. Endometrial biopsy was taken from the fundal area by means of endometrial biopsy curette and tissue obtained was preserved in 40% formalin.

Prepration Of Tissue For Histopathological Examination :

The tissue was processed through varying concentrations of alcohol; then cleared by passing through xylol. Blocks were made by embedding it in molten paraffin which was allowed to set. The sections

were then cut and fixed before staining with Ehrlich S hematoxylin and eosin stain as described by ***Liliequist*** (1953).

The slides were examined under low power and high power, and endometrium was phased. Finally, findings of ultrasound and histopathology were correlated.

Observations

OBSERVATIONS

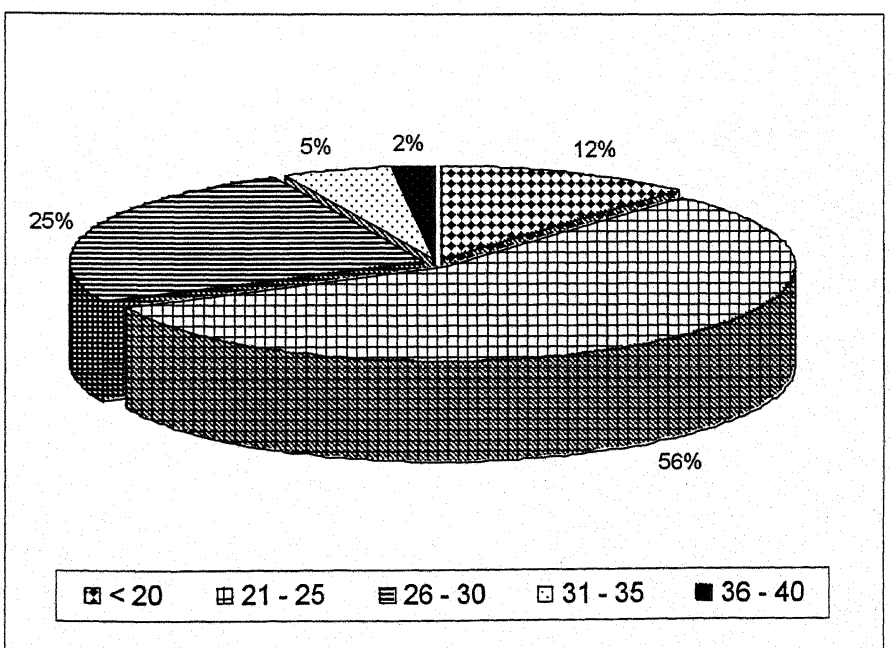
The present study was carried out in the Department of Obstetrics and Gynaecology in conjunction with Department of Pathology M.L.B. Medical College, Jhansi.

A total of 100 patients with primary infertility underwent this study.

After taking detailed history all cases underwent a thorough clinical examination, transvaginal ultrasound and endometrial biopsy. The observations were as follows :-

TABLE I :- DISTRIBUTION OF CASES ACCORDING TO AGE

Age Group (In years)	*Study Group*	
	Number	*Percentage (%)*
< 20	12	12
21 – 25	56	56
26 – 30	25	25
31 – 35	5	5
36 – 40	2	2



Age of the patients is our study ranged from 18 to 40 years. Table 1 shows the distribution of cases according to the age. In the study group maximum number of cases were in the age group of 21 – 25 years (56 % cases), followed by 26 – 30 years (25% cases) and 18 -20 years (12% cases). Two cases were above 35 years of age. No case below 18 years was included as vaginal sonography cannot be done is these cases.

Table II shows distribution of cases according to socio– economic status. Most of the cases (50%) belonged to group III (low) socio-economic status and (46%) in middle class. Only (4%) of the patients belonged to group I or high class.

TABLE II :- DISTRIBUTION OF CASES ACCORDING TO SOCIOECONOMIC STATUS

Socioeconomic Status	*Study Group*	
	Number	*Percentage (%)*
I (High)	4	4
II (Middle)	46	46
III (Low)	50	50

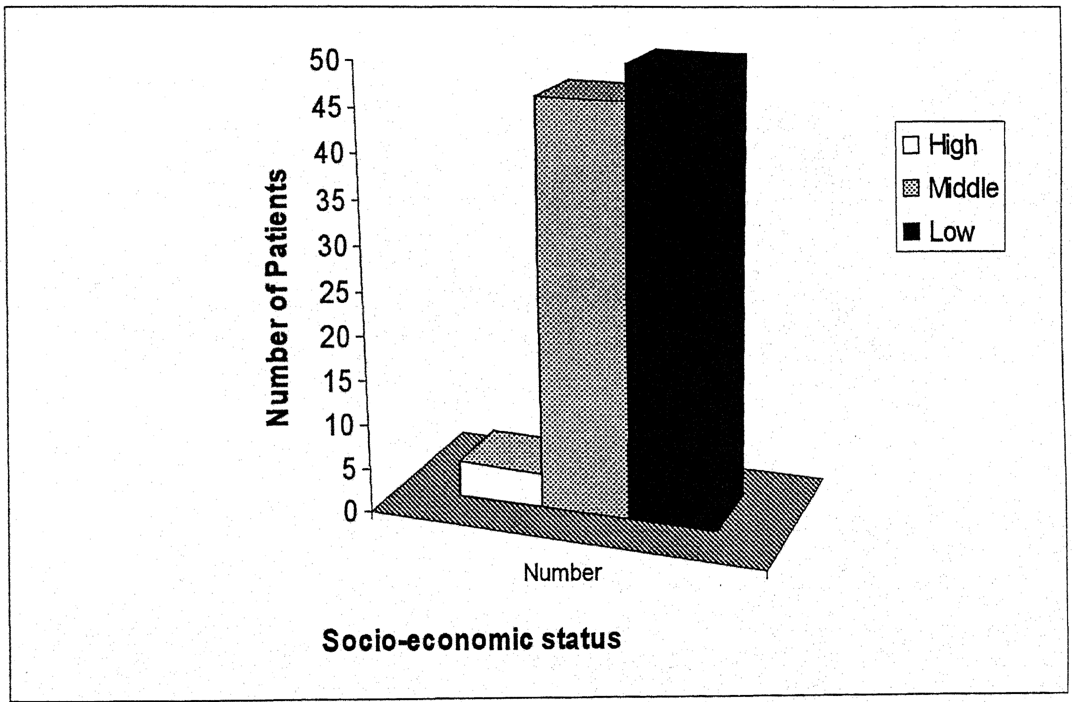
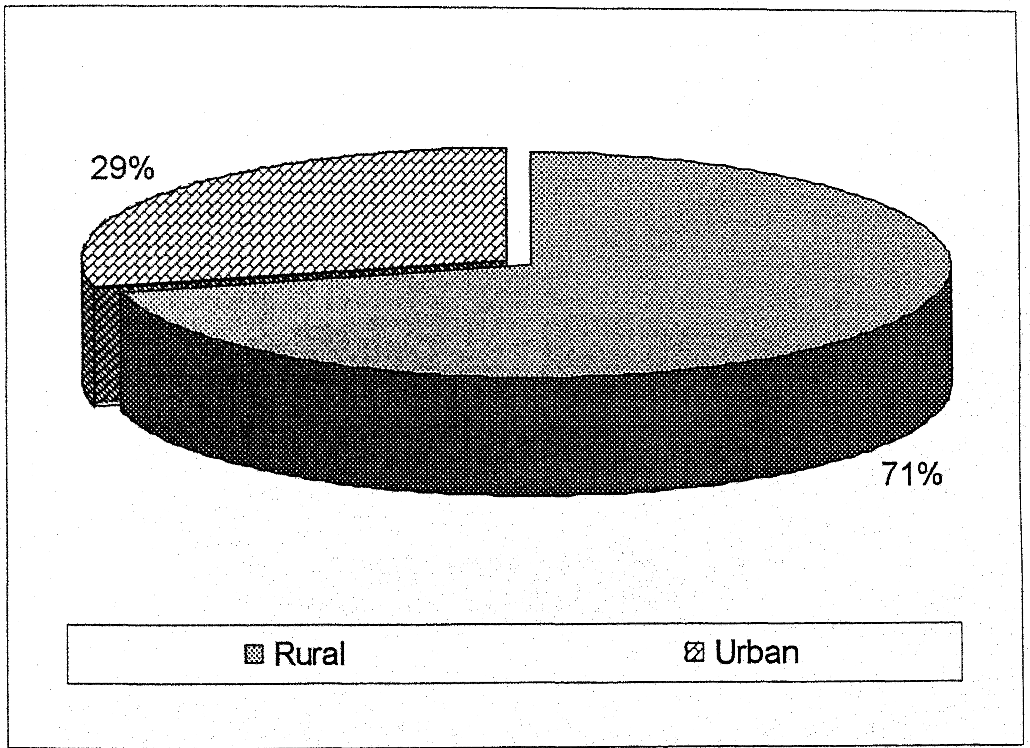


TABLE III :- DISTRIBUTION OF CASES ACCORDING TO RESIDENTIAL AREAS

Residing Area	***Study Group***	
	Number	***Percentage (%)***
Rural	71	71
Urban	29	29



This table shows that maximum of 71% cases in the study group were residing in rural areas. Only 29% patients were residents of urban areas.

TABLE IV :- DISTRIBUTION OF CASES ACCORDING TO EDUCATION STATUS

Education Status	*Study Group*	
	Number	*Percentage (%)*
Illiterate	29	29
Undergraduate	59	59
Graduate	5	5
Postgraduate	7	7

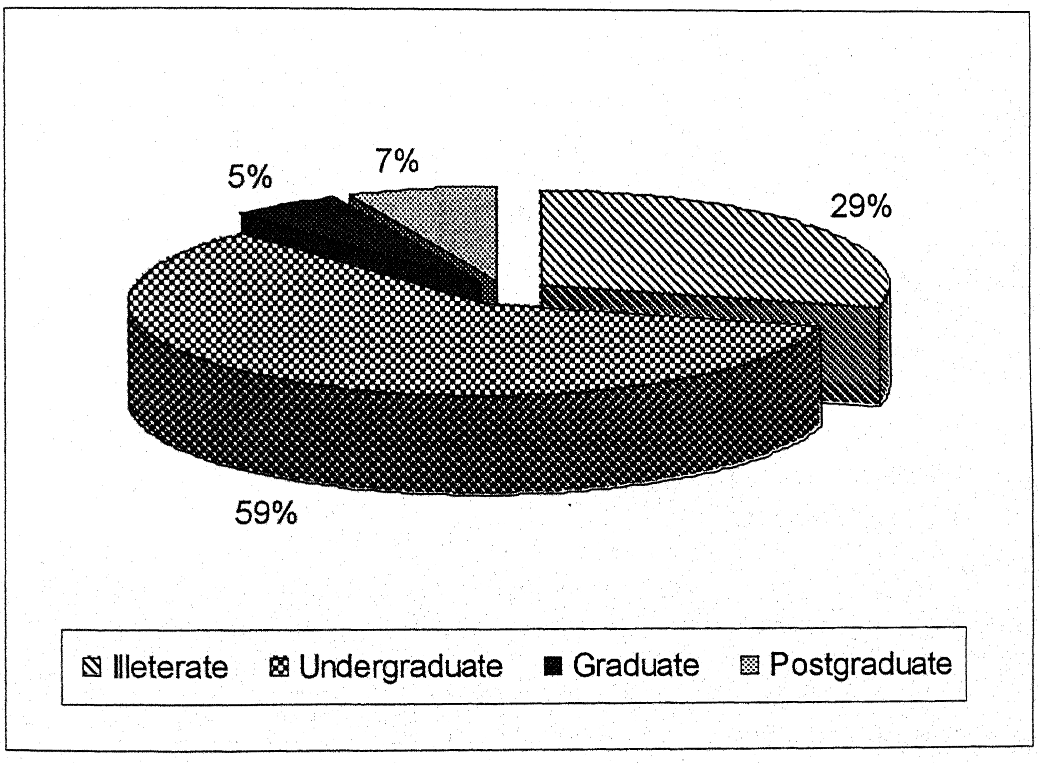
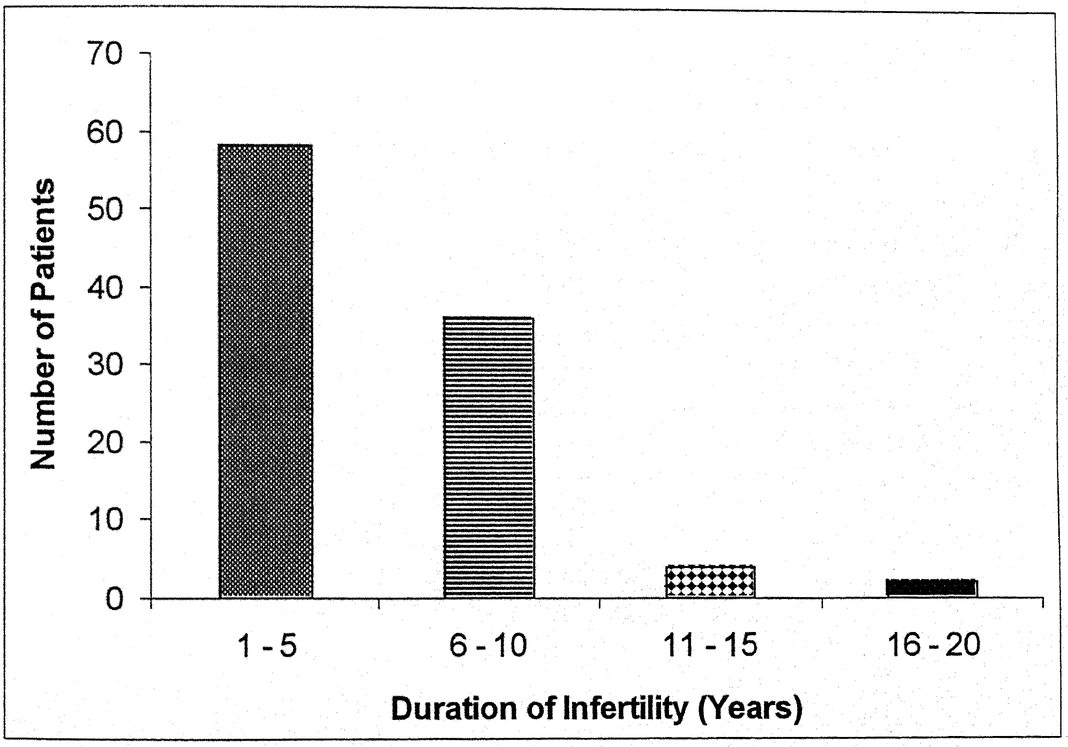


Table IV shows that according to educational status most of the patients (59%) were undergraduates, followed by (29%) who were illiterate. Only 5% of the patients were graduates and 7% were postgraduates.

TABLEV :- DISTRIBUTION OF CASES ACCORDING TO DURATION OF INFERTILITY

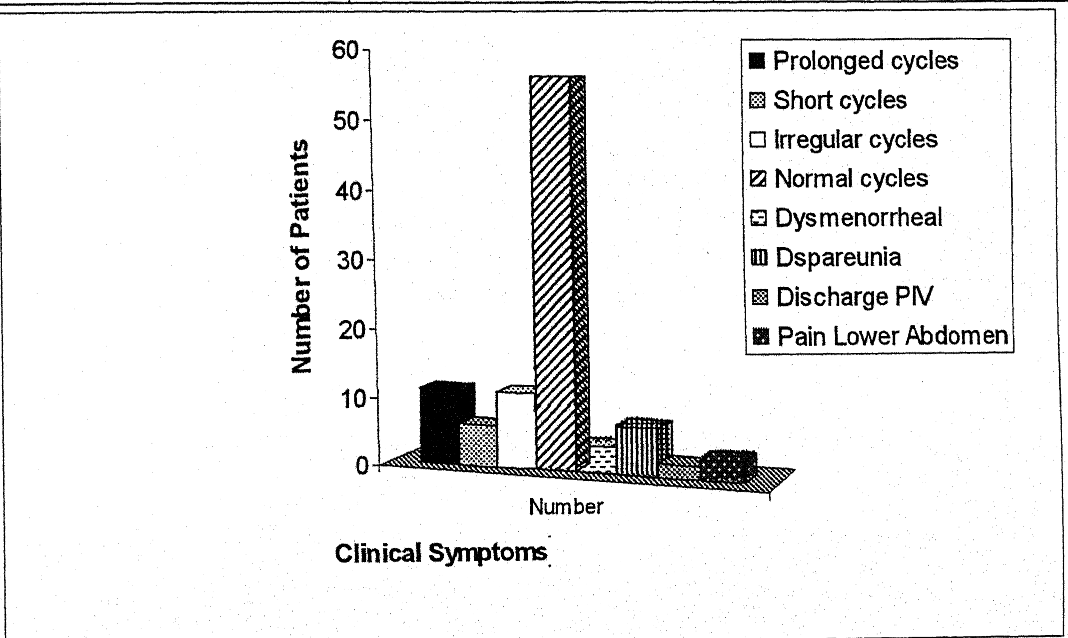
Duration of Infertility (Years)	*Study Group*	
	Number	*Percentage (%)*
1-5	58	58
6-10	36	36
11-15	4	4
16-20	2	2



This table shows the distribution of cases according to their duration of infertility. Maximum number of patients in the study group (58%) had 1-5 years of period of infertility; followed by (36%) of patients will 6-10 years duration, and in only (2%) cases, duration of infertility was 16-20 years.

TABLE VI :- DISTRIBUTION OF CASES ACCORDING TO CLINICAL SYMPTOMS

Clinical Symptoms	*Study Group*	
	Number	*Percentage (%)*
Prolonged cycles	11	11
Short cycles	6	6
Irregular cycles	11	11
Normal cycles	56	56
Dysmenorrhoea	4	4
Dypareunia	7	7
Discharge P/V	2	2
Pain lower abdomen	3	3



Out of 100 cases most of the patients (56%) had normal menstrual cycles. In the rest of cases, prolonged cycles were present in 11% ; irregular cycles in 11% cases and short cycles in 6% cases. Dsypareunia was present in 7% cases. 4% cases had dysmenorrhoea; 3% had pain lower abdomen; and 2% had complaints of discharge per vaginum.

TABLE VII :- DISTRIBUTION OF CASES ACCORDING TO THE SIZE OF UTERUS (CLINICAL ASSESSMENT)

Size of Uterus	*Study Group*	
	Number	*Percentage (%)*
Normal	75	75
Hypoplastic/Small	17	17
Enlarged/Bulky	8	8

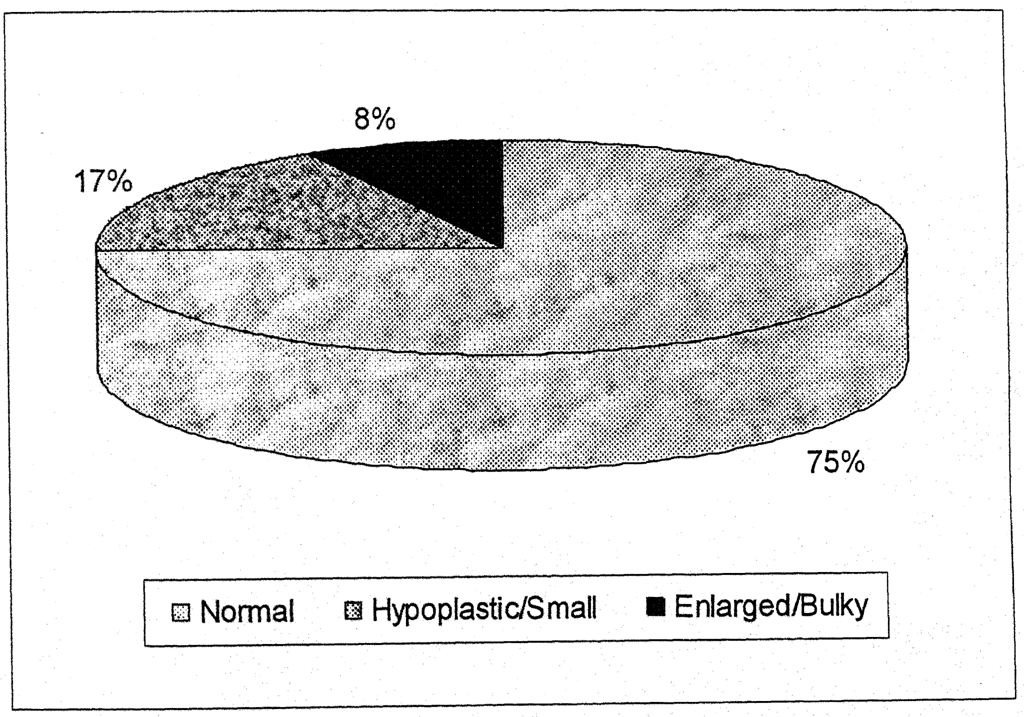


Table VII shows distribution of cases according to the size of uterus, assessed by bimanual examination. In the study group, 75% cases had normal sized uterus, 17% cases had small or hypoplastic uterus; and only 8% cases had enlarged or bulky uterus. Out of the 17% cases with small uterus, 1 case had prolapse uterus. Out of the cases having bulky uterus, 2 cases on TVS showed fibroid uterus. In all the cases, adenexa were found to be normal.

TABLE VIII :- MYOMETRIAL CHARACTERS BY TVS

Myometrial characters	*Study Group*	
	Number	*Percentage (%)*
Echotexture		
Homogenous	98	98
Heterogenous	2	2
Contraction		
Present	44	44
Absent	56	56

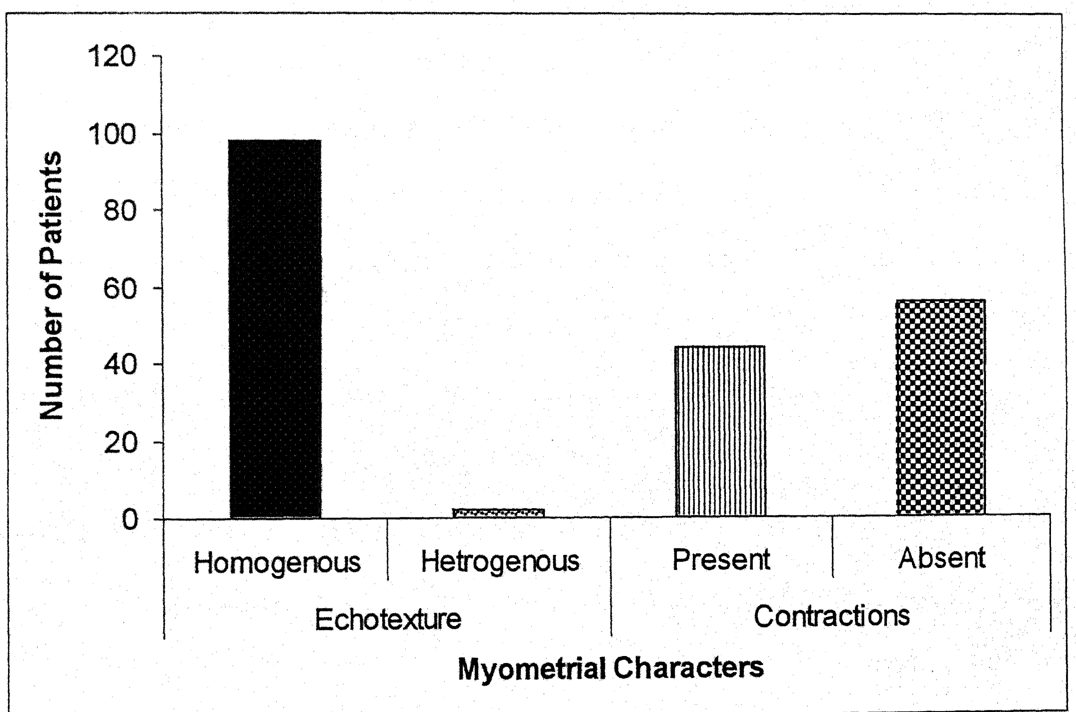


Table VIII shows myometrial characters seen with transvaginal songraphy. The characters studied were echotexture of myometrium and myometrial contractions. Myometrium was found to be of homogenous echotexture in maximum (98%) cases. This is a normal feature of myometrium. Only 2% cases had heterogenous echotexture, and these cases further on were found to have fibroid uterus.

Myometrial contractions were absent in most of the cases i.e. 56%. This is characteristic of secretory phase in which uterus becomes quiescent in preparation for implantation. 44% cases showed presence of myometrial contractions.

Table IX shows various endometrial characters as seen by TVS.

Endometrium was studied for its echogenecity, contractions, triple-line appearance and vascularity.

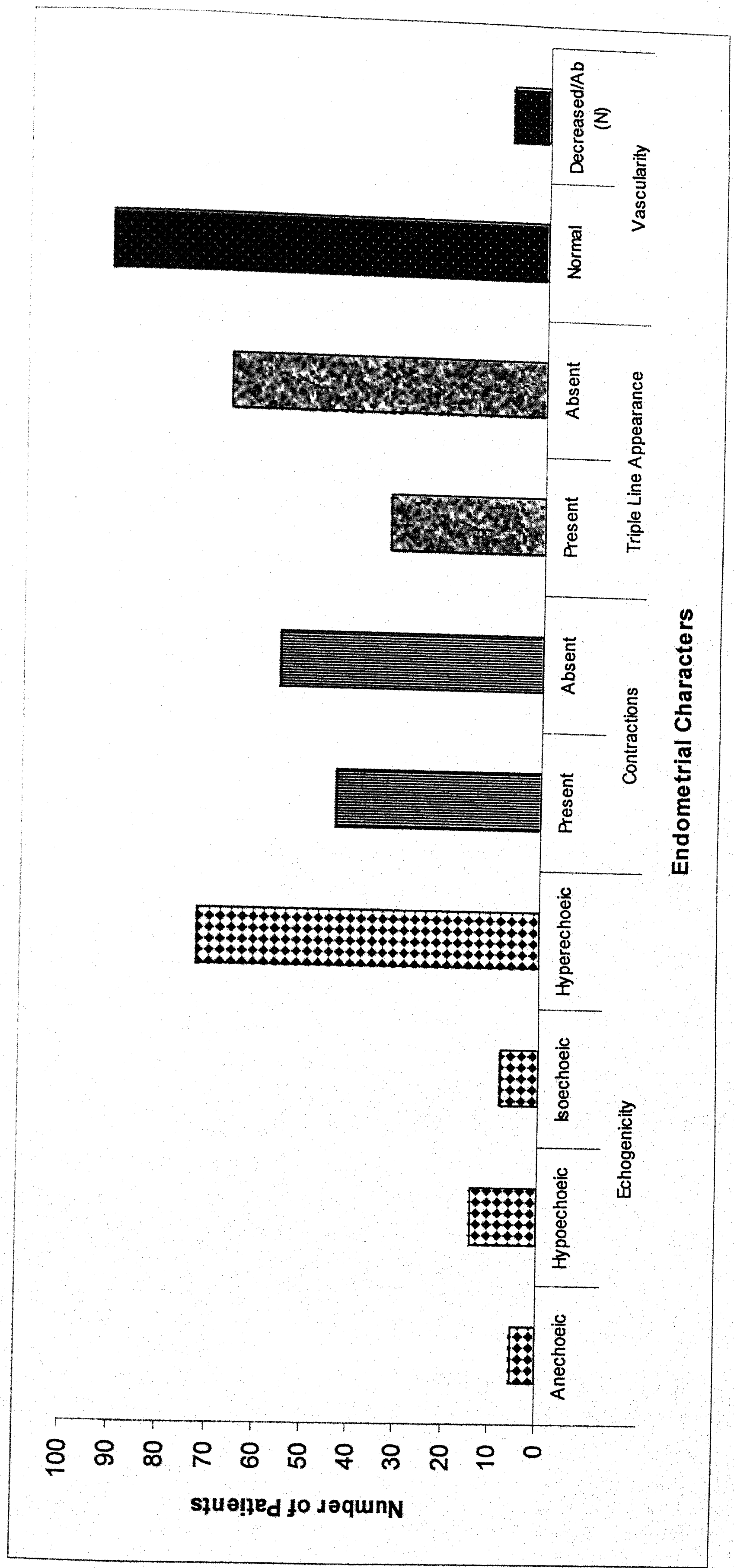
Hyperechoic endometrium was present in maximum (73%) cases and such type of endometrium is characteristic of secretory phase. 14% of cases had hypoechoeic endometrium, and 8% had isoechoeic endometrium. These two types characterize early and late proliferative phase. Only in 5% cases, the endometrium was anechoic.

Endometrial contractions, which are in true sense transmitted myometrial contractions were absent is 56% cases and present in 44% cases. Triple-line appearance which is a characteristic feature of early proliferative or follicular phase was seen in only 33% of cases, and was absent in 67% of cases; which means that most of the cases had secretory endometrial transformation. Normal vascularity inside the

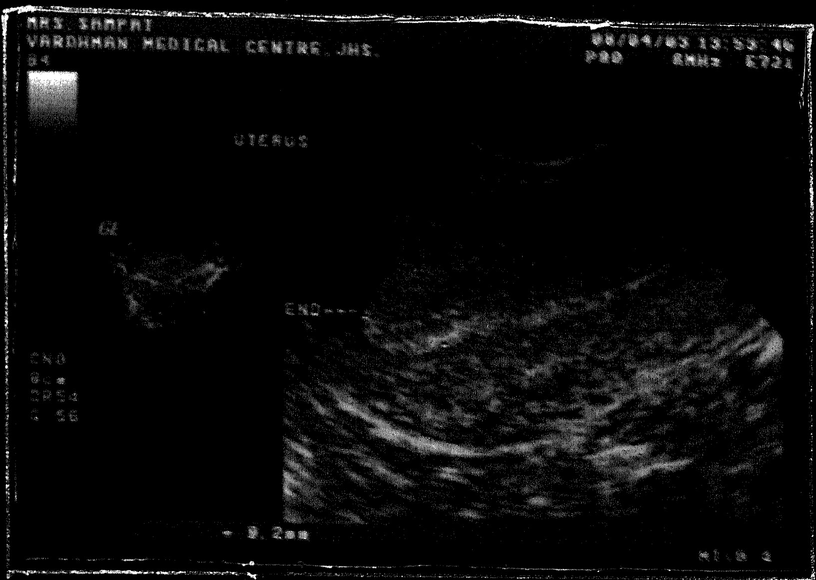
uterus, also called as cold uterus was seen in 92% cases. Only 8% cases showed decreased vascularity.

TABLE IX :- ENDOMETRIAL CHARACTERS BY TVS

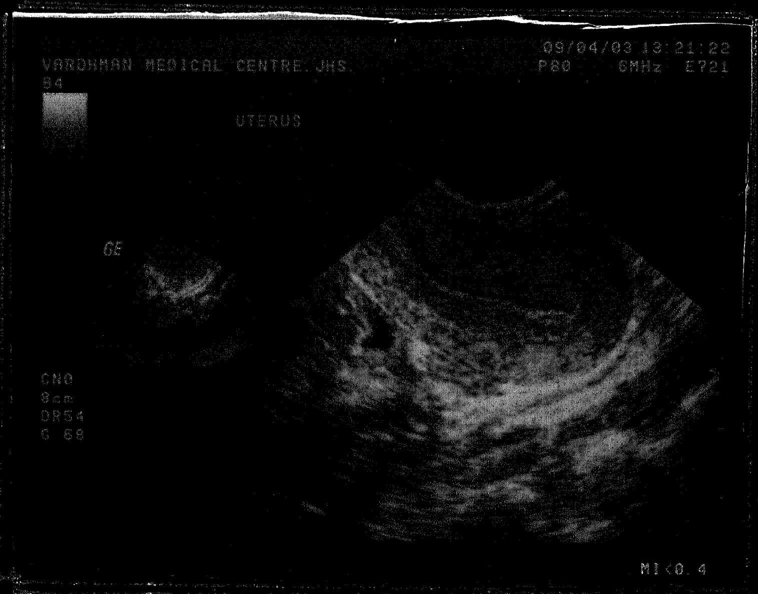
Endometrial characters	*Study Group*	
	Number	*Percentage (%)*
Echogenecity		
Anechoeic	5	5
Hypoechoeic	14	14
Isoechoeic	8	8
Hyperechoeic	73	73
Contractions		
Present	44	44
Absent	56	56
Triple Line Appearance		
Present	33	33
Absent	67	67
Vascularity		
Normal	92	92
Decreased/Ab (N)	8	8



Number of Patients
100
90
80
70
60
50
40
30
20
10
0
Anechoic
Hypoechoic
Isoechoic
Hyperechoic
Echogenicity
Present
Absent
Contractions
Present
Absent
Triple Line Appearance
Normal
Decreased/Ab (N)
Vascularity
Endometrial Characters



SECRETORY PHASE ECHOGENIC ENDOMETRIUM MEASURING 9.2MM; CHARACTERISTIC OF PREMENSTRUAL PHASE.



EARLY PROLIFERATIVE PHASE ENDOMETRIUM SHOWING TRIPLE-LINE APPEARANCE, CHARACTERISTIC OF THIS PHASE.

TABLE X :- ENDOMETRIAL THICKNESS BY TVS

Endometrial Thickness (In mm)	*Study Group*	
	Number	*Percentage (%)*
0 – 3	0	0
3 – 6	6	6
6 – 9	34	34
9 – 12	37	37
12 – 15	21	21
15 – 18	1	1
18 – 21	1	1

Mean $\pm$ S.D. = 9.9 $\pm$ 2.9mm

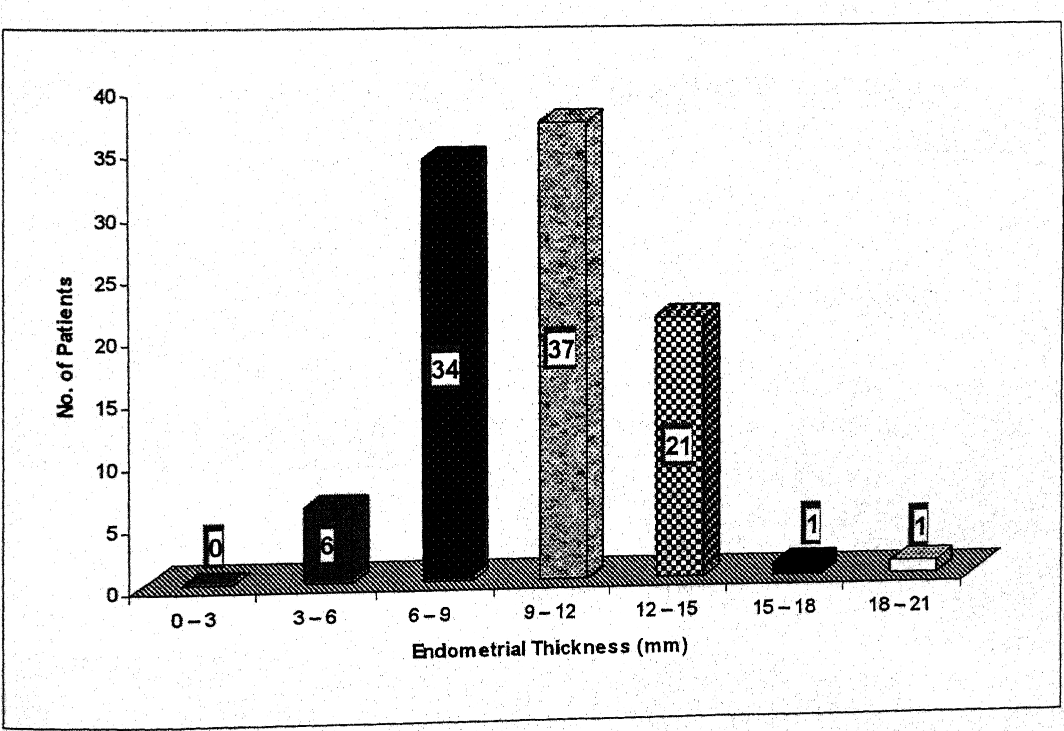


Table X shows that in maximum number of cases i.e. 37% had endometrial thickness in the range of (9-12) mm, followed 34% cases in the range (6-9) mm. 21% of cases had endometrial thickness ranging from (12-15) mm featuring late secretory transformation. 6% of cases showed endometrium in (3-6) mm range; and only 1% cases each in (15-18) mm and (18-21) mm range.

The mean thickness of endometrium in 100 cases studied was found to be 9.9mm $\pm$ 2.9mm standard deviation.

Table XI shows that TVS of endometrium done in premenstrual phase revealed that most of the cases i.e. 65% showed secretory or ovulatory phase, and 32% showed proliferative or anovulatory phase. Out of 65% showing secretory transformation, 37% cases revealed late secretory transformation and 28% had early secretory changes. Of the 32% cases showing proliferative transformation, 16% had late proliferative changes, and rest 16% had early proliferative changes.

3% of the cases showed intrinsic abnormalities of the uterus. Of these, 2% cases had fibroid uterus (as also assessed by bimanual examination); and 1 case showed presence of endometrial polyp.

TABLE XI :- ENDOMETRIAL PHASE BY TVS

Endometrial Phase	*Study Group*	
	Number	*Percentage (%)*
Anovulatory		
Early Proliferative	16	16
Late Proliferative	16	16
Ovulatory		
Early Secretory	28	28
Late Secretory	37	37
Intrinsic abnormalities		
Fibroid uterus	2	2
Endometrial Polyp	1	1

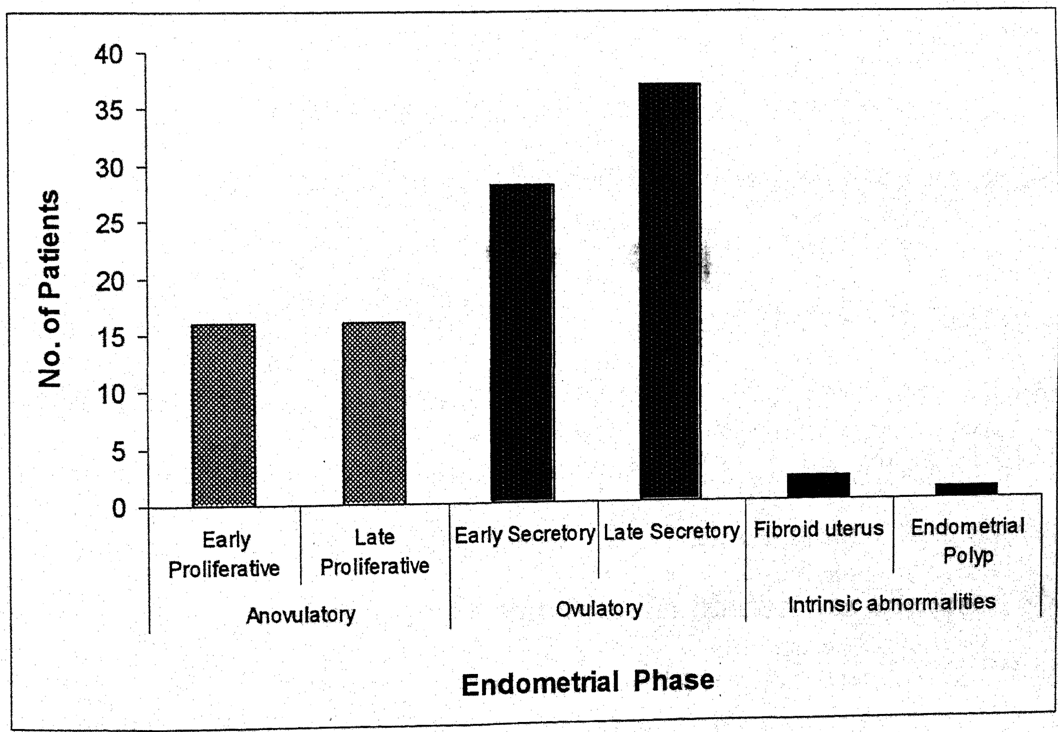
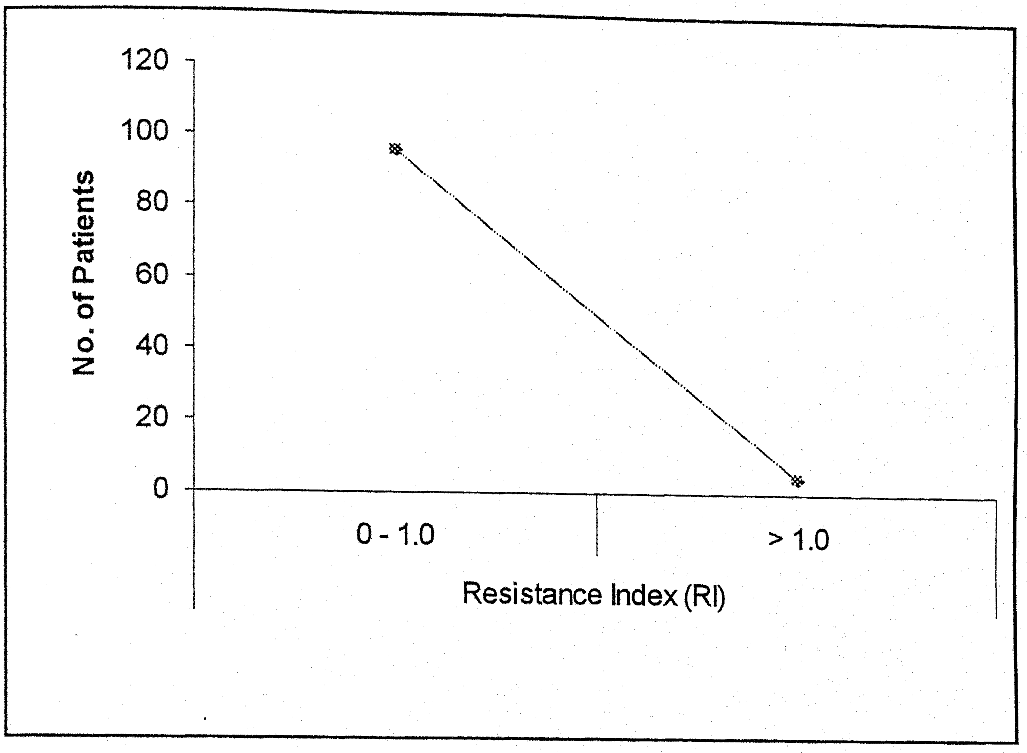
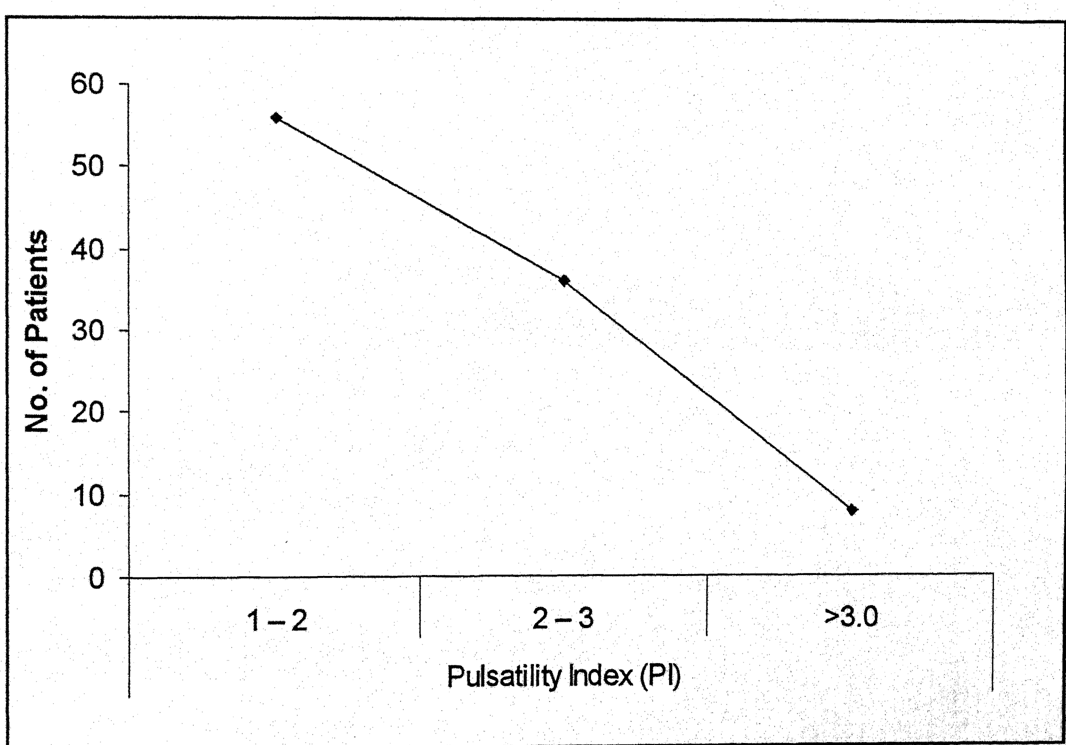


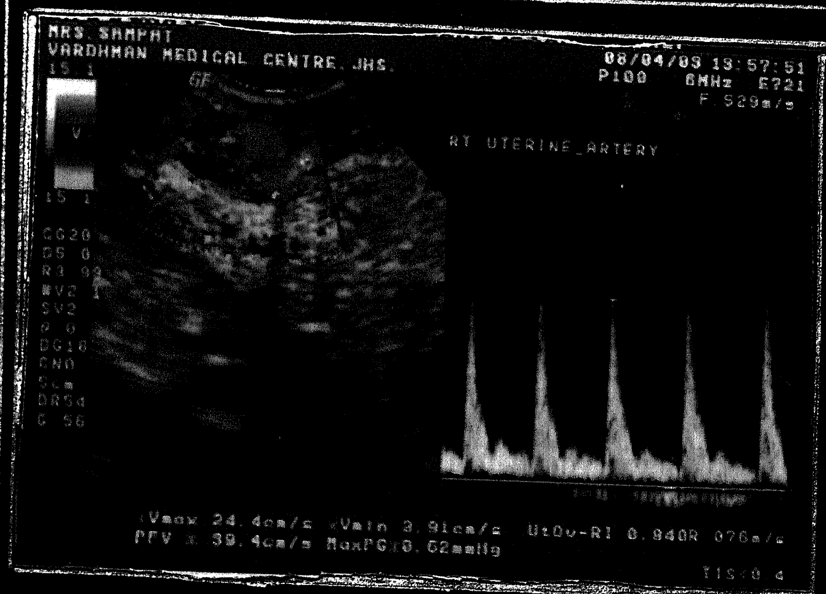
TABLE XII :- UTERINE ARTERY FLOW CHARACTERS BY TVS COLOUR DOPPLER

Flow Parameters	*Study Group*	
	Number	*Percentage (%)*
Resistance Index (RI)		
0 - 1.0	96	96
> 1.0	4	4
Pulsatility Index (PI)		
1 – 2	56	56
2 – 3	36	36
>3.0	8	8

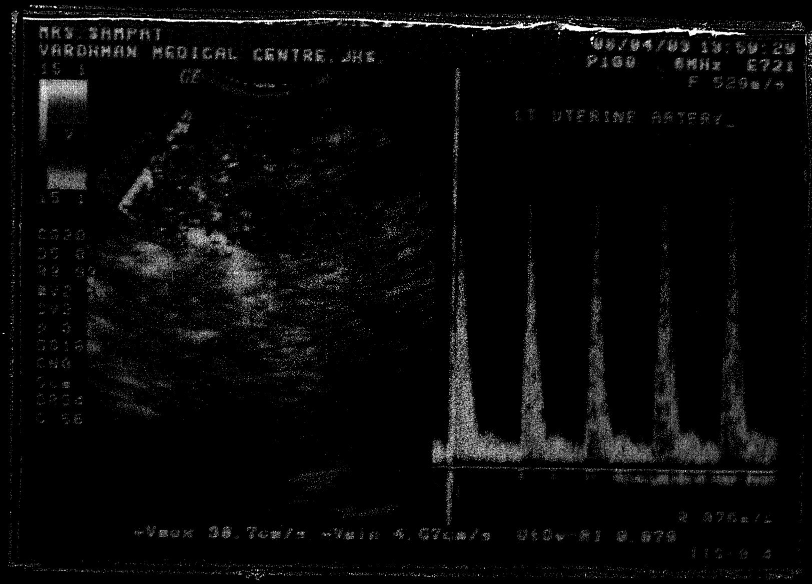
Table XII shows the study of uterine artery doppler flow parameters like Resistance or Pourcelot Index (RI) and Pulsatility Index (PI). In the study group, most of the cases (96%) showed resistance Index in the range of 0-1.0, which is a normal feature. Only 4% cases showed RI >1.0, which signifies that there was either decreased or no flow in the uterine artery during the diastolic phase of cardiac cycle. Out of 100 cases, 56% showed pulsatility Index (PI) in the range of (1-2) followed by 36% cases in the range (2-3); which means that uterine artery flow during both systolic and diastolic phases was adequate. 8% cases showed PI value >3.0, which again denotes decreased blood flow during diastolic phase.


120
100
80
60
40
20
0
No. of Patients
0 - 1.0
> 1.0
Resistance Index (RI)


60
50
40
30
20
10
0
No. of Patients
1 – 2
2 – 3
>3.0
Pulsatility Index (PI)

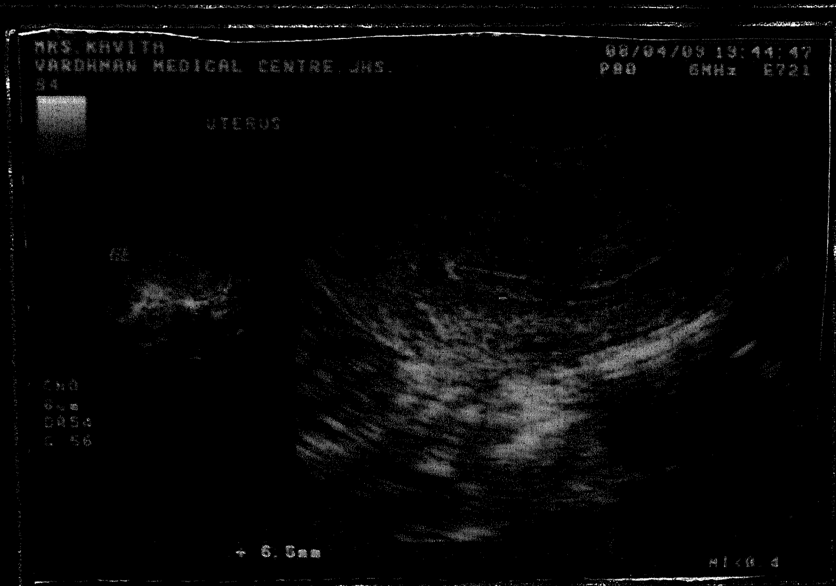


WAVEFORM ANALYSIS SHOWING GOOD END-DIASTOLIC FLOW IN RIGHT UTERINE ARTERY DURING PREMENSTRUAL PHASE.

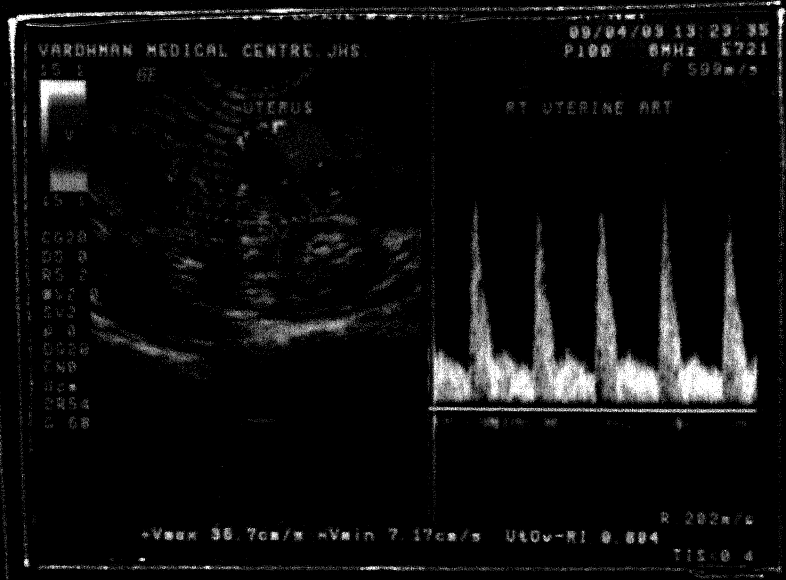


WAVEFORM ANALYSIS SHOWING GOOD END-DIASTOLIC FLOW IN LEFT UTERINE ARTERY DURING PREMENSTRUAL PHASE.

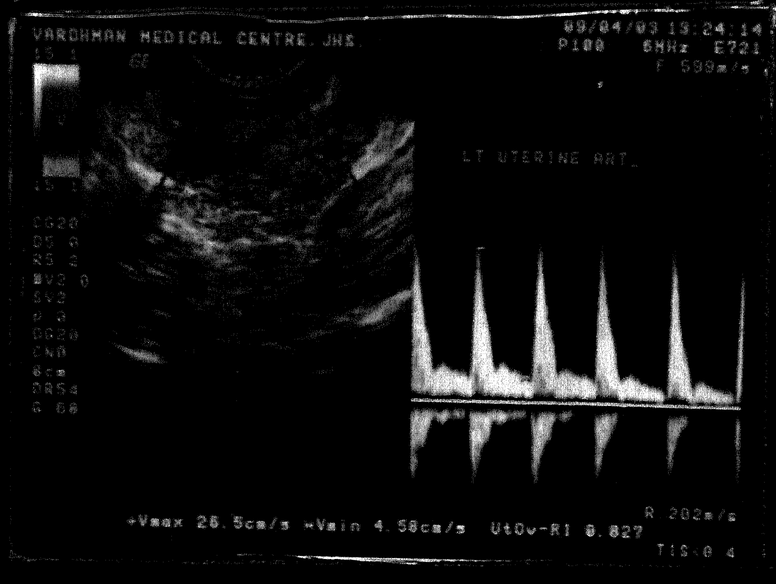
WAVEFORM ANALYSIS SHOWING ABSENT DIASTOLIC FLOW IN LEFT UTERINE ARTERY.



PROLIFERATIVE PHASE HYPOECHOEIC ENDOMETRIUM MEASURING 6.5MM. THE THICKNESS IS LESS THAN THAT EXPECTED IN PREMENSTRUAL PHASE. THIS IS DUE TO ABSENT DIASTOLIC FLOW IN THIS PHASE.



WAVEFORM ANALYSIS SHOWING GOOD END-DIASTOLIC FLOW IN RIGHT UTERINE ARTERY DURING SECRETORY PHASE.



WAVEFORM ANALYSIS SHOWING GOOD END-DIASTOLIC FLOW IN LEFT UTERINE ARTERY DURING SECRETORY PHASE.

HISTOPATHOLOGICAL CHANGES CHARACTERISTIC OF PROLIFERATIVE PHASE WERE SEEN IN PREMENSTRUAL PHASE.

HISTOPATHOLOGICAL CHANGES CHARACTERISTIC OF SECRETORY PHASE WERE SEEN IN PREMENSTRUAL PHASE.

Table XIII shows that normal secretory endometrium was present in 63% cases, and proliferative endometrium in only 33% cases as seen in histopathological slides of endometrial biopsy. Endometrial hyperplasia was present in 2% cases, 1% of which had simple endometrial hyperplasia, and 1% had benign cystic hyperplasia. 2% cases showed features of tubercular endometritis.

Table XIV shows that patients who had regular cycles showed secretory phase in 40 cases, and proliferative phase in 7 cases. Patient who presented with chief complaints of menorrhagia had proliferative endometrium in 9 cases, normal secretory endometrium in 7 cases, and simple hyperplasia is 1 case. Patients with polymenorrhoea showed proliferative endometrium in 7 cases, and secretory endometrium in 2 cases. Likewise proliferative and secretory endometrium and tubercular endometritis were present in 3,11, and 2 cases respectively in patients whose chief complaint was oligomenorrhoea.

Patients with acyclical bleeding had proliferative, secretory and benign cystic hyperplasia is 8, 2 and 1 cases respectively.

TABLE XIV :- CORRELATION OF MENSTRUAL PATTERNS WITH HISTOPATHOLOGY OF ENDOMETRIUM.

Histopathological Findings	*Study Group*				
	Menorrhagia	*Polymenorrhoea*	*Oligome-norrhoea*	*Acyclical*	*Regular*
Proliferative Phase	9	7	3	8	7
Secretory Phase	7	2	11	2	40
Simple Hyperplasia	1	-	-	-	-
Benign Cystic Hyperplasia	-	-	-	1	-
Tubercular Endometritis	-	-	2	-	-

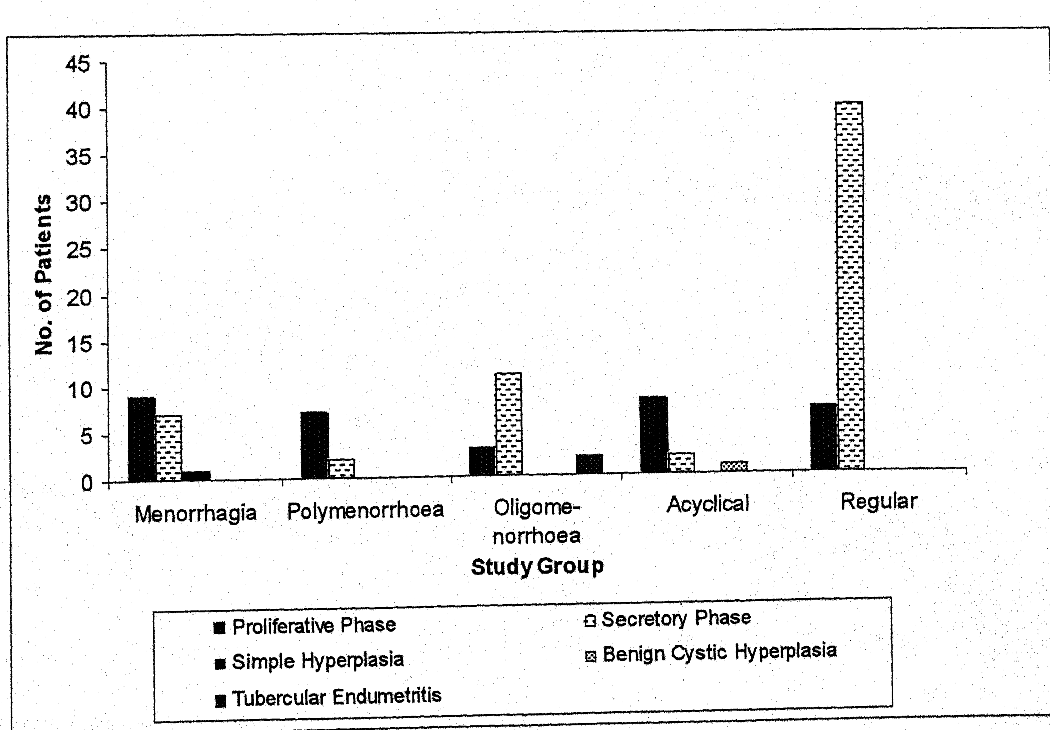


TABLE XV :- CORRELATION OF FINDINGS OF TVS WITH HISTOPATHOLOGY OF ENDOMETRIUM.

Histopathological findings	*Study Group*				
	I	*II*	*III*	*IV*	*Total*
Proliferative	25	3	7	2	37
Secretory	3	-	40	16	59
Benign Cystic Hyperplasia	-	1	-	-	1
Simple Hyperplasia	1	-	-	-	1
Tubercular Endometritis	-	-	1	1	2
Total	29	4	48	19	100

Anovulatory: I - Early Proliferative; II - Late Proliferative

Ovulatory: III - Early Secretory; IV – Late Secretory

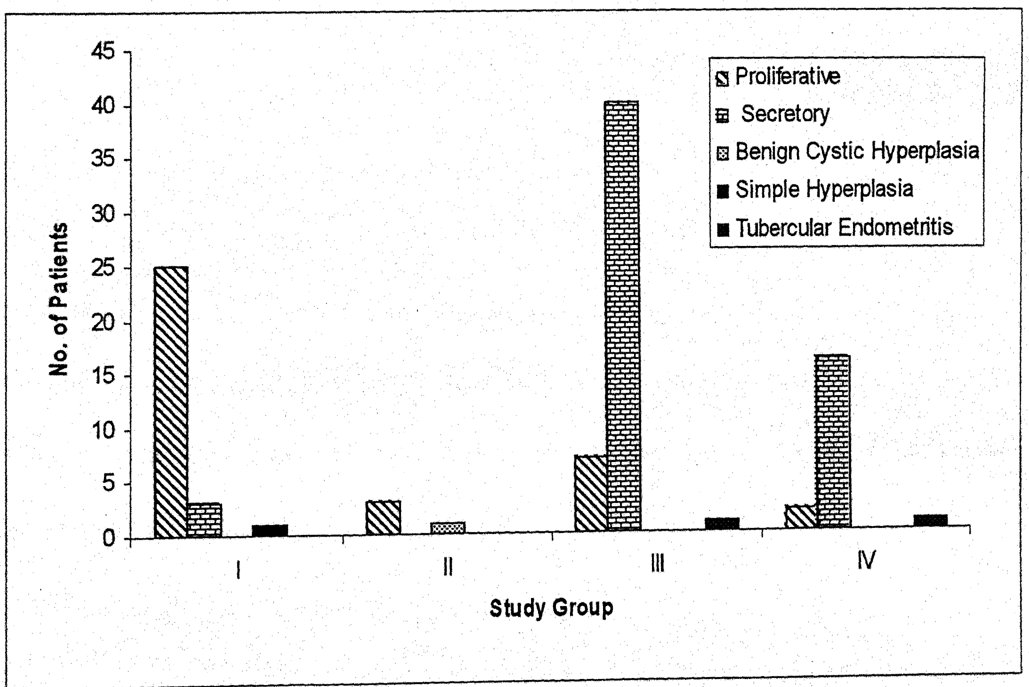


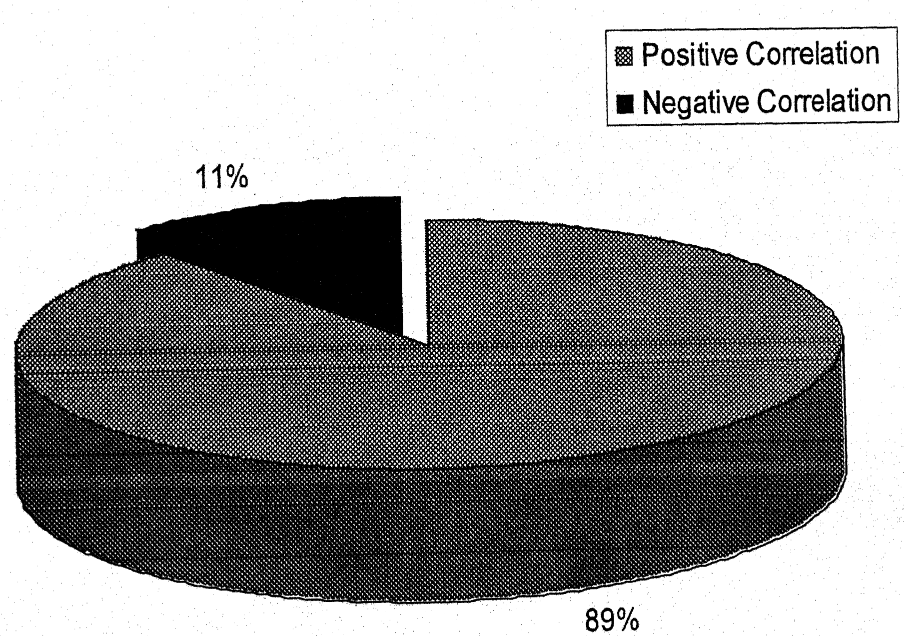
Table XV shows correlation of findings of TVS with histopathology of endometrium in primary infertility cases. Out of 29 cases who revealed Type I endometrium on TVS, proliferative endometrium was present is 25 cases, while 3 cases had secretory endometrium, and 1 case showed simple hyperplasia on histopathology. Out of 4 cases, who showed Type II endometrium on TVS revealed proliferative changes in 3 cases and benign cystic hyperplasia in 1 case in histopathological study.

Out of 59 cases showing secretory changes in histopathology, 40 cases had Type III endometrium, 16 had Type IV endometrium and only 3 cases had Type I endometrium. One case which showed benign cystic hyperplasia on histopathology revealed Type II endometrium with TVS. Of the 2 cases which showed tubercular endometritis on histopathology, one had Type III endometrium, and other had Type IV endometrium on TVS.

Table XVI shows that in case of primary infertility the diagnosis by transvaginal ultrasound and histopathology matched in 89% cases; while in 11% cases there was a disparity among the two modes of investigation. On histopathology, out of 11 cases, 7 cases which showed secretory phase on TVS were found to have proliferative changes; 2 cases which showed proliferative phase on TVS were found to have secretory changes; and the remaining 2 cases showed tubercular endometritis instead of secretory phase as seen with TVS.

TABLE XVI :- HISTOPATHOLOGICAL CORRELATION IN PRIMARY INFERTILITY

Correlation	*Positive Correlation*		*Negative Correlation*	
	No.	%	No.	%
Transvaginal Sonography	89	89	11	11



Discussion

DISCUSSION

Ever since the birth of Couise Browne (25th July' 78), when infertility was conquered by IVF-ET, various developments have been made in the field of human fertilization. The permanent inability to achieve good pregnancy rates inspite of fertilization rates as high as 100%, has been harvesting researchers. That it was implantation which limited the success of IVF, was ever realized it was proposed that embryo quality is the key factor in implantation. Inspite of best quality embryos the implantation results have remained same. Of late, it was suggested that there is something inherent in which decides the increased implantation of the blastoscyst, the "uterine receptivity".

At this point of time, the problem being faced by specialists is how to predict uterine receptivity successfully. At first, microscopic and ultramicroscopic studies of endometrial biopsies were pioneered, but because of invasiveness and harm to embryo in peri-ovulatory period and mid-luteal phase newer research dimensions proposed doppler ultrasonography to predict uterine receptivity. Using this modality, both the endometrial morphology as well as uterine blood flow can be assessed. By far, most of the work has been done in ART'S.

Lately, ***Goswamy's*** hypothesis, ***"impaired uterine perfusion"*** a cause of infertility has extended this research in patients of unexplained infertility. Uterine receptivity in cases of infertility during natural cycles has been studied by a few workers only; our study was one of such kinds.

Science and technology are advancing at a very rapid pace and so are the ultrasound machines and probes. The advancements have led to improved resolution, clearer pictures, better understanding of the physiology, blood flow and perfusion of organs.

Historically, TVS has developed from Kratrochwill first attempting to put a probe into vagina with a specially designed chair to today, when we have finger tip probes, finger cap probes and multifrequency, multidirection probes.

TVS, by using transducer with greater frequency and because of its proximity to genital organs, can study uterus and endometrium in greater detail and with more accuracy. So it has proved to be of great help in excluding pathologies not detectable by physical examination.

In the present study, 100 cases of primary infertility have been taken. After taking detailed history and performing a complete clinical examination, transvaginal colour doppler ultrasonography was performed followed by histopathological study of endometrium in premenstrual phase.

In our study maximum number of cases were is the age group of 21 – 25 years. (Table I)

Name	***Years***	***Incidence in reproductive age group***
Hull et al	1985	17%
Nyberg et al	1985	10%
Eisenberg et al	1986	10 – 15%
Daniel et al	1989	10-15%
Templeton et al	1990	14%

According to the study done by ***Tan et al*** (1992), ***Templeton et al*** (1996), ***Sherfield et al*** (1993), ***Lipitz et al*** (1994), the most important single factor influencing infertility is the female age.

Navot et al (1991) and ***Edward S. et al*** (1991) concluded the main factor responsible for declining infertility with increasing age is the oocyte quality, rather than endometrial receptivity.

Most of the patients in our study belonged to low socio-economic status, with maximum number of patients residing in rural areas.

59% of patients in our study group were undergraduates, followed by 29% who were illiterate. With increasing literacy status, familiarity with advanced diagnostic and investigatory facilities increases. This was reflected by the fact that, although few cases in our study (7%) who were postgraduates were more aware and regular in their diagnostic work up, as compared to the illiterate group.

Maximum number of cases in the study group (58%) had 1-5 years of duration of infertility. Our results match with the study conducted in the University Clinic at Kandang kerbau Hospital Singapore between 1980 to 1983, in which the mean duration of infertility was 3.2 years. No case with less than 12 months of duration of marriage were included, as these cases fall outside the definition of infertility. From these 12 months of duration, period during which contraception was used, was excluded.

As is obvious from our study (Table VI), most of the patients had normal menstrual cycles (56%), followed by prolonged cycles (11%), irregular cycles (11%), short cycles (6%). Dysmenorrhoea, dyspareunia, pain lower abdomen and discharge per vaginum were among the other complaints

Ovulatory dysfunction occurs is 30-40% of infertile patients. Most patients with anovulation present with irregular or abnormal menses or amenorrhoea. A normal menstrual cycle is a sensitive and reliable barometer of hypo-thalamo-pitutary function.

The fact that cycles are normal in most patients suggests that infertility in such cases could be accounted for by other factors, excluding ovulatory dysfunctions.

In the present study, size of uterus, when assessed clinically was found to be normal in 75% cases; and small or hypoplastic in 17% cases. The small size mainly corresponded to the presence of irregular menstrual patterns or scanty menses in such cases. One case had prolapse uterus; and 2 cases of bulky uterus revealed the presence of fibroid, with TVS. Both prolapse and fibroid uterus are among the important causes of infertility.

SONOGRAPHIC FINDINGS

The accuracy of ultrasound in analysis of disorders of the female pelvis has been found to be between 82% and 91% by ***Cochrane W.J. et al*** (1974), ***Fleischer et al*** (1978), ***Lawson T. L. et al*** (1977), ***Walsch J.W. et al*** (1975).

The uterus is a pear shaped organ with relatively low level echoes, measuring less than 8 cms, with long axis (from base of cervix to the top of fundus), less than 5.5 cms in width and less than 3 cms in antero-posterior dimensions (***Green B,*** 1980). The normal uterine canal, corresponding to the apposed endometrial linings is usually thin and bright, extending upto the midline of uterus and surrounding a relatively echo free endometrium (***Sample et al*** 1980). The endometrial echo can

be imaged in almost 100% of cases, and is typically less bright than the midline vaginal echo.

The thickness and brightness of the endometrial lining, however are not constant, but vary throughout the menstrual cycle; and during the secretory phase (post ovulation), the lining is usually slightly thicker and more echogenic than during proliferative phase.

MYOMETRIAL CHARACTERS WITH TVS

The myometrium consists of smooth muscles and on ultrasound, this appears as a homogenous structure of medium echodensity. The echotexture of myometrium was found to be homogenous in maximum number of cases (98%). 2% cases who showed heterogenous texture were found to have intramural fibroid uterus; and in these cases, this could be the cause of infertility.

Myometrial contractions occur as local or focal contractions as well as contraction waves that propagate from the cervix towards the fundus or vice versa. Prior to ovulation, these contraction waves move towards the fundus; and after ovulation, frequency of contractions decreases in prepration for implantation. Myometrial contractions were absent is most (56%) of the cases, which is a characteristic feature of secretory phase (Table VIII).

ENDOMETRIAL CHARACTERS BY TVS

Endometrium was studied for its echogenecity, contractions, triple-line appearance, its thickness and vascularity.

The echocharacteristics of endometrium and endometrial canal should be important in those uterine pathological conditions which mainly involve the uterine endometrium.

Hyperechoeic endometrium was found in most cases (73%). These findings correspond to those of ***Smith et al's*** (1984) Grade A endometrium, which characterizes the luteal / postovulatory period.

Endometrial contractions were absent is most cases (56%). ***Birnholtz et al*** (1984) reported that the endometrial contractions were most common in the follicular phase and culminate around ovulation. Moreover, contractions were towards the fundus at this time which aids in sperm transport and maintainence of pregnancy.

Oike and collegues (1990) correlated endometrial contractions with hormonal levels. They found that endometrial peristalsis had strong correlation with estradiol levels.

Triple-line-appearance, characteristic of early proliferative phase was seen in only 33% cases, and was absent in 67% cases.

The interface between the two leaves of endometrium is the central echogenic line. The two outer lines represent the basilis of the developing endometrium. The endometrium appears anechoeic compared with these reflections – ***Gonen and Casper et al*** (1990).

Normal vascularity inside the uterus was seen in maximum number of cases (92%). Uterine perfusion is largely dependent on patients age, phase of menstrual cycle and other specific conditions. The spiral arteries undergo substantial anatomical changes during the menstrual cycle. The increased endometrial vascularity is highly dependent upon the uterine, arcuate and radial artery blood flow. Blood

flow velocity waveform changes in the spiral arteries during normal ovulatory cycles are characterized by low velocity ($p < 0.05$) and lower impedance to blood flow ($p < 0.05$), than are those observed in uterine arteries with larger diameter (***Kupesic et al***, 1993).

In our study, endometrial thickness in most (37%) cases was in the (9-12) mm range, followed by 34% cases in the range of (6-9) mm.

Endometrial thickness is measured through the central longitudinal axis of the uterine body (***Gonen*** 1990).

Endometrial thickness in conception and non-conception cycles.

Authors	**Conception cycles (mm)**	**Non-conception cycles (mm)**
Fleischer et al (1984)	10	10
Thickman et al (1986)	7.4	8.8
Gonen et al (1989)	8.7	7.5
Welker et al (1989)	6-16	No difference
Check et al (1991)	> 10	< 10
Cassidenti et al (1993)	10.6	10.9
Our study	-	9.9 ± 2.9

Mean thickness in our study was 9.9mm. ± 2.9mm. S.D., which corresponds most closely with findings of ***Fleisher et al*** (1984).

TYPE OF ENDOMETRIUM WITH TVS

In the present study on primary infertility patients, the echogenecity of endometrium was variable. 32% cases showed hypoechogenic endometrium, representing the proliferative phase. Out of these, 16% showed three - layered endometrium; and in the rest 16%, endometrium became more echogenic, probably related to the

development, enlargement and tortuosity of endometrial glands and to numerous interfaces that arise from distended and tortuous glands representing periovulatory period. There was a hypoechoeic area within the inner endometrium producing ring-like structure which most likely represents oedema of compactum layer. This occurs concurrently with rise in progesterone, and represents earliest sign of ovulation. 65% cases had shown progressive increase in the relative endometrial/myometrial echogenecity from endometrial base towards the surface; and out of these 65% cases, 37% had shown completely hyperechogenic endometrium. They probably represent secretory phase (Table XI).

UTERINE ARTERY DOPPLER FLOW PARAMETERS

The doppler parameters included in our study were pulsatility Index (PI) and resistance Index (RI). In the study group, most of the cases (96%) showed resistance index in the range of 0-1.0, which is a fairly favourable feature.

PI in 92% cases was < 3.0, which again favours normal blood supply during both phases of cardiac cycle.

In anovulatory cycles, a continuous increase of the uterine artery RI has been detected. Moreover, in some infertile patients, end-diastolic flow is absent. The uterine artery flow could be used to predict a hostile uterine environment prior to embryo transfer (***Steer et al*** 1991).

Estrogen and progesterone receptors are present is human uterine arteries. The receptor content of arterial walls is highest just before ovulation. It is particularly interesting that the lowest blood flow impedance occurs at the time of peak luteal function (RI 0.84 $\pm$ 0.04),

during which time implantation is most likely to occur. It is logical that blood supply to the uterus should be high in the luteal phase, as has been reported by ***Kurjak et al*** (1991), ***Goswamy et al*** (1988), ***Steer et al*** (1991), ***Battalgia et al*** (1990). Our results of decreased blood flow impedance during luteal phase are consistent with findings of aforementioned workers.

Scholtes et al (1989) recorded the highest value for pulsatility index in the uterine arteries on cycle day 16. This was explained by increased uterine contractility and compression of the vessels traversing the uterine wall; which decreases their diameter and consequently cause higher resistance to flow.

ENDOMETRIAL PATTERNS ON HISTOPATHOLOGY

In the present series of 100 cases, histopathological, study of endometrium revealed normal secretory change in 60% cases, and proliferative changes is 36% cases, endometrial hyperplasia in 2%, and tubercular endometritis in remaining 2% cases.

ENDOMETRIAL PATTERN ON HISTOLOGY

Endometrial change	***Shetty et al (1959)***	***Gupta et al (1980)***	***Jadhav and Raichur et al (1987)***	***Krishna mohan et al (1993)***
I) Anovulatory a) Proliferative (including deficient and intermediate proliferation)	14.1	16.9	25.0	7.5
b) Irregular proliferation and hyperplasia	11.1	5.9	-	2.5
II) Ovulatory a) Adequate secretory response	74.8	60.4	75.0	80.0
b) Deficient secretory response	-	8.1	-	7.5
III) Intrinsic abnormalities a)Tubercular endometritis	-	8.7	-	-
b) Endometrial polyp	-	-	-	-
c) Others (Endometritis)	-	-	-	2.5

Our results correspond with the study done by Gupta et al (1980).

Endometrial dating by Novak and Woodruff et al (1974) showed that the ideal time of EB is from 24the to 26the day of cycle i.e. in the premenstrual phase.

CORRELATION OF TVS AND HISTOPATHOLOGY

In the present study, there is significant correlation of endometrial characters between transvaginal doppler findings and histopathological findings. Out of total 29 cases who showed Type I endometrium on TVS, proliferative changes were present in 26 cases on histopathology.

Out of 4 cases, who should Type II endometrium on TVS, 3 cases revealed proliferative changes on histopathology.

Out of 59 cases showing secretory changes on histopathology, 40 cases had Type III endometrium on TVS, 16 cases had Type IV endometrium, and only 3 cases had Type I endometrium. One case which showed benign cystic hyperplasia on histopathology revealed Type II endometrium on TVS (Table XV).

In our study, diagnosis in cases of primary infertility by TVS and histopathology correlated in 89% cases; while in 11% cases there was negative correlation among the two procedures. On histology, out of 11 cases, 7 cases which showed secretory phase on TVS were found to have proliferative changes, 2 cases which showed proliferative phase on TVS revealed secretory changes on histopathology; and the remaining 2 cases showed tubercular endometritis instead of secretory phase, as seen with TVS.

According to ***Sahmay S. et al*, *Oral E et al*, *Saridogan E. et al*, *Atasu T et al*** (1995), EB not only shows the hormonal response of endometrium, it also gives information about local factors concerning atrophy, specific and non-specific infections and malignancy. In several series is which endometrial biopsies were performed on infertile women the incidence of performing a biopsy during a conception cycle ranged from 1.2 to 4%. The risk of disrupting the pregnancy by causing trauma and bleeding at implantation site ranges from 4% to 20%. Therefore the major disadvantage with EB is that it is an invasive procedure. Moreover, the results are not immediately available. In cases of infertility, whereas TVS can screen other pelvic organs as well for related pathologies in a single scan of few minutes, EB does not provide any information regarding myometrial and adenexal pathologies or vascular characters.

Thus, TVS can play a major role in diagnosis and management of primary infertility, as it has a greater patient compliance with non-invasive technique and without necessiating full bladder. It can very well substitute EB in the diagnostic work up of female infertility.

Summary and Conclusions

SUMMARY AND CONCLUSIONS

The present study "Role of Transvaginal colour doppler in cases of primary Infertility as compared with endometrial biopsy" was carried out in the post graduate department of obstetrics and gynaecology, MLB, MC, Jhansi.

A total of 100 cases of primary infertility were studied. The observations are summarized herewith.

(1) In our study, patients were in the reproductive age group i.e. 18-40 years. Maximum number of cases i.e. 56% belonged to age group 21-25 years.

(2) Most of the patients is our study belonged to low socio-economic strata. The prevelance of infertility does not differ significantly among racial and ethnic groups. Although, patients seeking treatment for infertility are predominantly of high socio-economic strata; infertility is more common among groups of relatively low socio-economic status ***(Novak et al)***. Most of the patients in our study (71%) were residing in rural areas.

(3) According to educational status, most of the cases (59%) were undergraduates, and (29%) were illiterate. Only (7%) of patients were postgraduates. Improved familiarity with, and access to infertility services among the affluent and better-educated patients accounts for their greater use of these medical resources.

(4) Maximum number of patients (58%) is our study had 1-5 years of period of infertility.

(5) Out of 100 cases, most of the patients (56%) had normal menstrual cycles. Among rest of the cases, prolonged cycles and irregular cycles were most common, accounting for 11% in each group, and short cycles were present in 6% cases. Few patients also has complaints of dyspareunia, dysmenorrhoea and discharge P/V.

(6) On clinical examination, uterus was found to be of normal size in 75% cases, hypoplastic in 17% cases, and 8% cases had enlarged or bulky uterus.

(7) The echotexture of myometrium was homogenous in maximum (98%) cases, which is a normal feature of myometrium. 2% cases who revealed heterogeneous character were found to have fibroid uterus. Myometrial contractions were absent is 56% cases. This quiescent nature of myometrium characterizes secretory phase of menstrual cycle.

(8) Study of endometrial characters with TVS showed the presence of hyperechoeic secretory endometrium in 73% cases. Among the rest, 14% had hypoechoeic endometrium, 8% had isoechoeic endometrium, and in 5% cases, endometrium was anechoeic.

Endometrial contractions, which in real sense are transmitted myometrial contractions were seen in only 44% cases. In the rest 56%; they were absent, a feature characteristic of secretory transformation.

Triple-line-appearance, a characteristic feature of early follicular or proliferative phase was seen is only 33% cases.

Normal vascularity inside uterus i.e. cold uterus was seen in 92% cases.

(9). Thickness of endometrium was variable according to the phase of menstrual cycle. Most of the cases (37%) showed endometrial thickness is the range of (9-12) mm, followed by 34% cases in the range of (6-9) mm. The thickness of endometrium gradually increases in the secretory phase. However, endometrial thickness > 14-16 mm is classified as being hyperplastic.

(10) On TVS, 65% cases showed that endometrium was in the secretory phase; and 32% cases showed endometrial characteristics of proliferative phase. Among the cases showing secretory transformation, 37% revealed late secretory changes and 28% had early secretory changes.

(11) Uterine artery doppler flow parameters like RI and PI, as studied with TVS colour doppler revealed that most of the cases (96%) showed resistance index (RI) in the range 0-1.0, which is a normal feature of ovulatory cycles. In anovulatory cycles, a continuous increase in the uterine artery RI has been detected. 92% cases showed Pulsatility index (PI) <3.0, which signifies that uterine artery flow during both systolic and diastolic phases was adequate. Studies of uterine blood flow is a non-invasive assay of uterine receptivity and provides valuable information on the pathophysiology of infertility.

(12) On histopathology of cases of infertility, normal secretory endometrium was present in 60% cases, and proliferative endometrium in 36% cases. Endometrial hyperplasia was present

is 2% cases, out of which one had benign cystic hyperplasia, and other had simple hyperplasia. Two cases revealed tubercular endometritis on histopathology as additional finding.

(13) On correlating the findings of TVS with histopathology, we found that most of the cases who showed Type I,II endometrium on TVS had proliferative endometrium on histopathology. Out of 59 cases showing secretory changes on histopathology, 56 cases had Type III, or Type IV endometrium, and only 3 cases had Type I endometrium on TVS.

(14) An additional finding of Tubercular endometritis was reported in 2 cases. Both of them showed secretory transformation on TVS.

(15) Only 7 cases which were suspected to have secretory changes on TVS had proliferative endometrium on histopathology. Two cases who were reported to have secretory changes on histopathology revealed proliferative endometrium on TVS.

(16) Transvaginal sonography was helpful in diagnosing 89% cases correctly, whose sonographic findings were well correlated with endometrial pathologies. So, TVS had 89% accuracy. A negative correlation with histopathology was seen in 11% cases.

Thus, we conclude that TVS is an accurate diagnostic tool for cases presenting with primary infertility. It is a non-invasive OPD procedure, not requiring full bladder. In addition to excluding organic lesions and other adenexal pathologies, endometrium can be studied in detail and fairly accurately.

TVS has correlated well with histopathology in diagnosing proliferative or secretory endometrium, endometrial hyperplasia or

atrophy. So it can nearly and accurately replace the invasive check curettage done for diagnostic purposes.

Today, to even think of an infertility unit without the facilities of transvaginal scanning is like walking into dark without a torch.

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Master Chart

MASTER CHART

S. N.	Age (Yrs)	Socio-economic status	Residence	Education	Period of Infertility (Yrs)	Clinical Symptoms	Uterus size (P/V)	Myometrial Characters by TVS		Endometrial Characters by TVS						Colour Dopler study of uterine A		Endometrial Biposy (Phase)	Co-relation B/W TVS and Histopathology
								Echogenecity	Contractions	Echogenecity	Contractions	Triple line appearance	Vascularity	Thickness (mm)	Phase	RI	PI		
1	22	M	R	UG	3	RG	N	N	A	HE	A	A	N	7.4	S	1.00	3.00	S	P
2	23	M	R	UG	7	RG	N	N	A	HE	A	A	N	7.9	S	0.80	2.00	S	P
3	27	M	U	G	3	RG	N	N	A	HE	A	A	N	12.0	S	0.80	2.10	S	P
4	25	L	R	UG	2	Dyspareunia	N	N	A	HE	A	A	N	11.2	S	0.80	1.90	S	P
5	32	M	U	G	15	Dyspareunia	N	N	P	IS	P	P	N	6.3	PR	0.74	1.70	PR	P
6	23	M	U	UG	5	Dysmenorrhoea	N	N	A	HE	A	A	N	9.8	S	0.60	1.80	S	P
7	25	L	R	UG	10	Prolonged	N	N	P	HO	P	P	N	6.0	PR	0.70	2.60	S	A
8	19	L	R	I	3	RG	Small	N	A	HE	A	A	N	10.2	S	0.74	2.30	S	P
9	23	M	R	UG	3	Prolonged	Bulky	N	A	HE	A	A	N	9.4	S	0.84	2.10	S	P
10	30	L	R	UG	10	Dyspareunia	N	N	A	HE	A	A	N	10.8	S	0.80	1.90	S	P
11	29	L	R	UG	4	Dyspareunia	Small	N	A	HE	A	A	N	11.4	S	0.80	1.80	S	P
12	30	L	R	I	5	Dyspareunia	Small	N	A	HE	A	A	N	9.0	S	0.89	2.80	S	P
13	22	M	R	UG	6	RG	N	N	A	HE	A	A	N	10.0	S	0.90	2.60	S	P
14	25	M	R	UG	8	RG	N	N	A	HE	A	A	N	12.2	S	0.76	2.40	PR	A
15	25	M	R	UG	10	Prolonged	Small	N	A	HE	A	A	N	12.1	S	0.80	2.20	S	P
16	25	H	U	G	2	Dyspareunia	N	N	P	HO	P	P	N	5.0	PR	0.90	2.80	Simple Hyperplasia Benign	P
17	20	L	R	UG	5	Irregular	N	N	A	HE	A	A	N	9.8	S	0.76	1.80	S	P
18	25	L	U	UG	10	Prolonged	Small	N	A	HE	A	A	N	10.2	S	0.80	1.90	S	P
19	20	M	R	I	4	RG	Small	N	A	HE	A	A	N	11.1	S	0.80	2.00	S	P
20	26	M	U	PG	6	RG	N	N	A	HE	A	A	N	11.2	S	0.76	2.00	S	P
21	27	M	U	PG	2	RG	N	N	A	HE	A	A	N	10.8	S	0.80	1.60	S	P
22	23	L	R	I	1.5	RG	N	N	A	HE	A	A	N	14.0	S	0.73	1.40	S	P
23	22	L	R	I	6	RG	N	N	A	HE	A	A	N	13.0	S	0.71	2.00	S	P

24	30	L	R	I	2	RG	Small	N	A	HE	A	A	N	12.8	S	0.72	2.00	S	P
25	21	M	U	G	4	RG	N	N	P	HO	P	P	N	5.4	PR	0.80	2.80	PR	P
26	25	L	R	UG	3	RG	N	N	P	HO	P	P	N	6.2	PR	0.90	2.60	PR	P
27	28	L	R	UG	10	RG	N	N	P	HO	P	P	N	4.6	PR	1.00	1.80	PR	P
28	33	M	U	UG	5	RG	N	N	P	IS	P	P	N	7.2	PR	0.60	1.90	PR	P
29	27	M	U	UG	10	RG	N	N	P	AN	P	P	N	6.8	PR	0.70	2.00	PR	P
30	28	M	R	UG	14	RG	N	N	P	HE	P	P	N	7.1	S	0.81	1.80	PR	A
31	21	M	R	UG	3	Prolonged	Small	N	P	HE	P	A	Ab (N)	10.0	S	1.00	3.30	S	P
32	18	L	R	I	3	RG	Small	N	P	AN	P	P	Ab (N)	7.6	PR	1.20	3.20	PR	P
33	24	M	R	UG	7	Dysmeno-rrhoea	N	N	A	HE	A	A	N	9.0	S	1.00	2.20	PR	A
34	18	L	R	I	1	RG	Bulky	N	P	AN	P	P	Ab (N)	6.6	PR	1.10	3.20	PR	P
35	35	M	R	UG	20	RG	Bulky	HT (Fibroid)	A	HE	A	A	N	8.1	S	0.82	1.99	S	P
36	30	M	R	UG	3	RG	N	N	P	HE	P	A	N	7.9	S	0.80	2.00	S	P
37	20	M	R	UG	3	Short	N	N	P	IS	P	P	N	8.1	PR	0.70	2.00	PR	P
38	25	M	U	UG	3	RG	N	N	P	AN	P	P	N	5.2	PR	0.81	2.10	PR	P
39	28	L	R	I	13	RG	N	N	P	HO	P	P	N	7.0	PR	0.82	1.90	PR	P
40	20	M	R	UG	2	Prolonged	N	N	P	HO	P	P	N	6.9	PR	0.76	1.60	PR	P
41	18	M	U	UG	3	Prolonged	N	N	P	IS	P	P	N	8.0	PR	0.76	1.80	PR	P
42	25	L	R	UG	3	RG	N	N	P	IS	P	P	Ab (N)	7.9	PR	1.30	3.20	PR	P
43	25	M	R	UG	4	Dysmeno-rrhoea	N	N	P	HE	P	A	Ab (N)	20.0	S	1.00	3.10	PR	A
44	20	L	R	UG	7	RG	N	N	P	HO	P	P	N	6.6	PR	1.00	2.00	PR	P
45	25	L	R	I	6	RG	N	N	P	IS	P	P	N	6.9	PR	0.80	2.10	S	A
46	26	L	R	I	6	RG	N	N	P	HE	P	P	N	7.6	PR	0.76	2.20	PR	P
47	25	L	R	UG	5	Prolonged	Small	N	P	IS	P	P	N	7.2	PR	0.78	1.90	PR	P
48	20	L	R	I	4	RG	Small	N	P	IS	P	P	N	7.6	PR	0.73	1.80	PR	P
49	20	L	R	I	3	Short	Small	N	P	HO	P	P	N	8.0	PR	0.80	1.70	PR	P
50	20	M	R	I	10	RG	N	N	P	HE	P	P	N	7.5	PR	0.82	1.90	PR	P
51	27	M	U	G	7	Irregular	N	N	P	HO	P	P	N	6.8	PR	0.81	2.00	PR	P
52	26	M	R	UG	4	RG	N	N	P	HE	P	A	N	14.4	S	0.80	2.20	S	P
53	22	M	U	UG	2.5	RG	N	N	P	HE	P	A	N	10.4	S	0.90	1.90	S	P
54	30	H	U	PG	7	Irregular	N	N	P	AN	P	P	N	7.1	PR	0.90	1.80	PR	P
55	30	M	R	UG	12	RG	N	N	P	HO	P	P	Ab (N)	6.5	PR	0.80	5.20	PR	P
56	23	M	U	UG	2	RG	N	N	P	HO	P	P	N	7.2	PR	0.78	2.80	PR	P
57	23	L	R	I	4	Prolonged	N	N	P	HO	P	P	N	7.1	PR	0.81	2.20	PR	P
58	23	L	R	UG	2	RG	N	N	A	HE	A	A	N	14.0	S	0.82	1.90	S	P

59	23	L	R	UG	10	RG	N	N	A	HE	A	A	N	16.0	S	0.78	1.80	S	P
60	35	M	R	UG	5	Dysmeno-rrhoea	Small	N	P	HE	P	A	N	9.0	S	0.90	2.10	PR	A
61	22	L	R	I	6	RG	N	N	A	HE	A	A	N	10.0	S	0.80	2.20	S	P
62	28	M	U	PG	2	Irregular	Bulky	N	A	HE	A	A	N	12.0	S	0.80	2.10	S	P
63	30	M	U	UG	5	RG	N	N	P	HE	P	P	N	9.0	S	0.75	2.00	Benign cystic Hyper-plasia	A
64	22	M	U	UG	5	RG	N	N	P	HE	P	A	N	10.8	S	0.84	2.40	S	P
65	22	L	R	UG	1.5	Short	N	N	A	HE	A	A	N	15.0	S	0.82	2.20	S	P
66	18	L	R	I	2	Irregular	N	N	A	HE	A	A	N	12.0	S	0.78	2.10	S	P
67	25	L	R	I	6	Irregular	N	N	A	HE	A	A	N	13.0	S	0.80	1.90	S	P
68	18	L	R	I	6	RG	N	N	A	HE	A	A	N	9.0	S	0.72	2.40	S	P
69	36	M	U	PG	6	RG	Small (Prola-pse)	N	A	HE	A	A	N	14.0	S	0.80	2.60	S	P
70	30	L	R	UG	8	RG	N	N	A	HE	A	A	N	10.0	S	0.90	2.80	S	P
71	28	L	R	UG	9	RG	Bulky	HT (Fibroid)	P	HE	P	A	N	11.7	S	0.80	2.70	S	P
72	30	M	U	PG	10	RG	Bulky	N	P	HO	P	A	Ab (N)	11.7	S	1.00	4.70	S	P
73	21	L	R	I	5	Prolonged	N	N	A	HE	A	A	N	12.4	S	1.00	2.00	S	P
74	25	L	R	I	10	Irregular	N	N	A	HE	A	A	N	13.8	S	1.00	2.10	PR	A
75	18	L	R	UG	7	Dyspareunia	Bulky	N	A	HE	A	A	N	14.2	S	0.90	2.00	S	P
76	25	L	R	I	10	Discharge P/V	N	N	A	HE	A	A	N	14.0	S	0.90	1.70	S	P
77	30	L	R	I	20	RG	N	N	A	HE	A	A	N	11.3	S	0.80	1.80	S	P
78	25	L	R	I	6	Pain lower abdomen	N	N	A	HE	A	A	N	10.6	S	0.80	1.90	S	P
79	24	L	R	UG	2	RG	N	N	A	HE	A	A	N	10.6	S	0.82	1.90	S	P
80	21	L	R	UG	2	Short	N	N	A	HE	A	A	N	11.8	S	0.76	1.90	S	P
81	18	L	R	UG	3	Irregular	N	N	P	HE	P	A	N	12.4	S	0.91	1.80	S	P
82	23	M	U	UG	4	Pain lower abdomen	Small	N	A	HE	A	A	N	12.2	S	0.69	1.80	S	P
83	25	L	R	I	8	Short	N	N	A	HE	A	A	N	11.8	S	0.90	1.90	S	P
84	20	L	R	I	8	Short	N	N	A	HE	A	A	N	10.9	S	0.86	2.10	S	P
85	23	M	U	UG	2	RG	N	N	A	HE	A	A	N	11.8	S	0.84	2.00	S	P

86	26	M	U	UG	10	RG	N	N	A	HE	A	A	N	11.2	S	1.00	2.40	TB endo-metritis	A
87	23	L	R	I	10	RG	Bulky	N	A	HE	A	A	N	11.8	S	0.80	2.30	S	P
88	30	M	U	PG	2	RG	N	N	A	HE	A	A	N	10.9	S	0.78	2.20	S	P
89	20	M	U	UG	3	Irregular	N	N	A	HE	A	A	N	14.6	S	0.78	2.00	S	P
90	18	L	R	UG	1.5	Irregular	N	N	A	HE	A	A	N	14.0	S	0.80	2.00	S	P
91	20	L	R	UG	1.5	RG	N	N	A	HE	A	A	N	12.8	S	0.82	1.90	S	P
92	18	M	U	I	3	Pain lower abdomen	N	N	A	HE	A	A	N	12.0	S	0.90	1.80	TB endo-metritis	A
93	18	M	U	UG	3	Discharge P/V	Small	N	A	HE	A	A	N	10.9	S	0.80	1.90	S	P
94	18	L	R	UG	3	RG	N	N	A	HE	A	A	N	10.8	S	0.90	1.80	S	P
95	24	L	R	UG	3	RG	N	N	P	HE	P	P	Ab (N)	5.0	PR	1.60	4.10	PR	P
96	23	L	R	I	8	Irregular	N	N	A	HE	A	A	N	10.0	S	0.80	2.10	S	P
97	22	M	R	UG	3	RG	N	N	A	HE	A	A	N	12.0	S	0.70	2.20	S	P
98	22	M	U	UG	3.5	Prolonged	N	N	P	HE	P	P	N	11.0	S	0.72	1.90	S	P
99	25	M	R	UG	10	RG	N	N	P	HE	P	P	N	7.0	PR	0.80	1.80	PR	P
100	20	M	R	UG	3	RG	Small	N	P	HE	P	A	N	13.7	S	0.74	1.50	S	P

M - Middle Class
U - Urban
I - Illiterate
RG - Regular
N - Normal
HT - Heterogenous
A - Absent
HE - Hyperechoeic
N - Normal
S - Secretory

L - Lower Class
R - Rural
UG - Undergraduate
P - Present
HO - Hypoechoeic
Ab(N) - Abnormal
PR - Proliferetive

H - High Class
G - Graduate
IS - Isoechoechoeic

PG - Postgraduate
AN - Anechoeic